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1. Your reference	JHC/P041074GB	
2. Patent application number *(The Patent Office will fill in this part)*	0307817.7	0 4 APR 2003
3. Full name, address and postcode of the or of each applicant *(underline all surnames)*	Trigen Limited 20 St James's Street LONDON SW1A 1ES	
Patents ADP number *(if you know it)*	7516081001	
If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom	
4. Title of the invention	BORONIC ACID COMPOUNDS	
5. Name of your agent *(if you have one)*	Harrison Goddard Foote	
"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Belgrave Hall Belgrave Street Leeds LS2 8DD	
Patents ADP number *(if you know it)*	~~44571001~~ 7631310002	

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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
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b) there is an inventor who is not named as an applicant, or
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Continuation sheets of this form	
Description	~~74~~ 75
Claim(s)	8
Abstract	-
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Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
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11. I/We request the grant of a patent on the basis of this applicati n.

Signature Date
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12. Name and daytime telephone number of person to contact in the United Kingdom Jonathan Couchman 0113 233 0100

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TITLE OF THE INVENTION

BORONIC ACID COMPOUNDS

BACKGROUND OF THE INVENTION

The present invention relates to pharmaceutically useful products obtainable from organoboronic acids. The application also relates to the use of members of the aforesaid class of products, to their preparation and formulation and to other subject matter.

Boronic Acid Compounds

It has been known for some years that boronic acid compounds and their derivatives, e.g. esters, have biological activities, notably as inhibitors or substrates of proteases. For example, Koehler et al. *Biochemistry* 10: 2477 (1971) report that 2-phenylethane boronic acid inhibits the serine protease chymotrypsin at millimolar levels. The inhibition of chymotrypsin and subtilisin by arylboronic acids (phenylboronic acid, m-nitro-phenylboronic acid, m-aminophenylboronic acid, m-bromophenylboronic acid) is reported by Phillip et al, *Proc. Nat. Acad. Sci. USA* 68: 478-480 (1971). A study of the inhibition of subtilisin Carlsberg by a variety of boronic acid, especially phenyl boronic acids substituted by Cl, Br, CH_3, H_2N, MeO and others, is described by Seufer-Wasserthal et al, *Biorg. Med. Chem.* 2(1): 35-48 (1994).

In describing inhibitors or substrates of proteases, P1, P2, P3, etc. designate substrate or inhibitor residues which are amino-terminal to the scissile peptide bond, and S1, S2, S3, etc., designate the corresponding subsites of the cognate proteinase in accordance with: Schechter, I. and Berger, A. On the Size of the Active Site in Proteases, *Biochem.Biophys.Res.Comm.*, **1967**, *27*, 157-162.

Pharmaceutical research into serine protease inhibitors has moved from the simple arylboronic acids to boropeptides, i.e. peptides containing a boronic acid analogue of an N-acyl-α-amino acid. The boronic acid may be derivatised, often to form an ester. Shenvi (EP-A-145441 and US 4499082) disclosed that peptides containing an α-aminoboronic acid with a neutral side chain were effective inhibitors of elastase and has been followed by numerous patent publications relating to boropeptide inhibitors of serine proteases. Specific, tight binding boronic acid inhibitors have been reported for elastase (K_i, 0.25nM), chymotrypsin (K_i, 0.25nM), cathepsin G (K_i, 21nM), α-lytic proteinase (K_i, 0.25nM), dipeptidyl aminopeptidase type IV (K_i, 16pM) and more recently thrombin (Ac-D-Phe-Pro-boro Arg-OH (DuP 714 initial K_i 1.2nM).

Claeson et al (US 5574014 and others) and Kakkar et al (WO 92/07869 and family members including US 5648338) disclose thrombin inhibitors having a neutral C-terminal side chain, for example an alkyl or alkoxyalkyl side chain.

Modifications of the compounds described by Kakkar et al are included in WO 96/25427, directed to peptidyl serine protease inhibitors in which the P2-P1 natural peptide linkage is replaced by another linkage. The aforesaid PCT application and its corresponding US patent (US 6127340) are included herein by reference, in particular the hydrophobic P3 and P2 residues described therein, the non-basic (hydrophobic) P1 residues described therein, and the described non-natural peptide linkages and their synthesis. As examples of non-natural peptide linkages may be mentioned $-CO_2-$, $-CH_2O-$, -NHCO-, $CHYCH_2-$, -CH=CH-, $-CO(CH_2)_pCO-$ where p is 1, 2 or 3, -COCHY-, $-CO_2-CH_2NH-$, -CHY-NX-, $-N(X)CH_2-N(X)CO-$, -CH=C(CN)CO-, -CH(OH)-NH-, -CH(CN)-NH-, $-CH(OH)-CH_2-$ or -NH-CHOH-, where X is H or an amino protecting group and Y is H or halogen, especially F. Preferred non-natural peptide linkages are $-CO_2-$ or $-CH_2O-$.

Metternich (EP 471651 and US 5288707, the latter being assigned to Trigen Limited) discloses variants of Phe-Pro-BoroArg boropeptides in which the P3 Phe is replaced by an unnatural hydrophobic amino acid such as TMSal, p-TBDPS –O-Me)-Phal or p-OH-Me-Phal and the P1 side chain may be neutral (alkoxyalkyl, alkylthioalkyl or trimethylsilylalkyl).

Amparo (WO 96/20698 and family members including US 5698538) disclose peptidomimetics of the structure Aryl-linker-Boro(Aa), where Boro(Aa) may be an aminoboronate residue with a non-basic side chain, for example BoroMpg. The linker is of the formula $-(CH_2)_mCONR-$ (where m is 0 to 8 and R is H or certain organic groups) or analogues thereof in which the peptide linkage -CONR- is replaced by -CSNR-, $-SO_2NR-$, $-CO_2-$, -C(S)O- or $-SO_2O-$. Aryl is phenyl, naphthyl or biphenyl substituted by one, two or three moieties selected from a specified group. Most typically these compounds are of the structure aryl $-(CH_2)_n-CONH-CHR^2-BY^1Y^2$, where R^2 is for example a neutral side chain as described above and n is 0 or 1.

Non-peptide boronates have been proposed as inhibitors of proteolytic enzymes in detergent compositions. WO 92/19707 and WO 95/12655 report that arylboronates can be used as inhibitors of proteolytic enzymes in detergent compositions. WO 92/19707 discloses compounds substituted *meta* to the boronate group by a hydrogen bonding group, especially acetamido ($-NHCOCH_3$), sufonamido ($-NHSO_2CH_3$) and alkylamino. WO 95/12655 teaches that *ortho*-substituted compounds are superior.

Boronate enzyme inhibitors have wide application, from detergents to bacterial sporulation inhibitors to pharmaceuticals. In the pharmaceutical field, there is ample patent literature describing boronate inhibitors of serine proteases, for example thrombin, faxtor Xa, kallikrein, elastase, plasmin as well as other serine proteases like prolyl endopeptidase and Ig AI Protease. Thrombin is the last protease in the coagulation pathway and acts to hydrolyse four small peptides form each molecule of fibrinogen, thus deprotecting its polymerisation sites. Once formed, the linear fibrin polymers may be cross-linked by factor XIIIa, which is itself activated by thrombin. In addition, thrombin is a potent activator of platelets, upon which it acts at specific receptors. Thrombin also potentiates its own production by the activation of factors V and VIII.

Other aminoboronate or peptidoboronate inhibitors or substrates of serine proteases are described in:

- US 4935493
- EP 341661
- WO 94/25049
- WO 95/09859
- WO 96/12499
- WO 96/20689
- Lee S-L et al, *Biochemistry* **1977**; *36*, 13180-13186
- Dominguez C et al, *Bioorg. Med. Chem. Lett.* **1977**; *7*, 79-84
- EP 471651
- WO 94/20526
- WO 95/20603
- WO97/05161
- US 4450105
- US 5106948
- US 5169841.

Peptide boronic acid inhibitors of hepatic C virus protease are described in WO 01/02424.

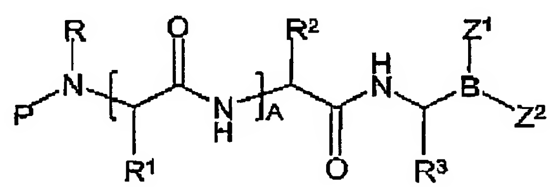
Boronic acid and ester compounds have displayed promise as inhibitors of the proteasome, a multicatalytic protease responsible for the majority of intracellular protein turnover. Ciechanover, *Cell*, **79**: 13-21 (1994), teaches that the proteasome is the proteolytic component of the ubiquitin-proteasome pathway, in which proteins are targeted for degradation by conjugation to multiple molecules of ubiquitin. Ciechanover also teaches that the ubiquitin-proteasome pathway plays a key role in a variety of important physiological processes.

Adams et al, US Patent No 5780454 (1998), US Patent No 6066730 (2000), US Patent No 6083903 (2000) and equivalent WO 96/13266, and US Patent No 6297217 (2001), hereby

incorporated by reference in their entirety, describe peptide boronic ester and acid compounds useful as proteasome inhibitors. The references also describe the use of boronic ester and acid compounds to reduce the rate of muscle protein degradation, to reduce the activity of NF-κB in a cell, to reduce the rate of degradation of p53 protein in a cell, to inhibit cyclin degradation in a cell, to inhibit the growth of a cancer cell, to inhibit antigen presentation in a cell, to inhibit NF-κB dependent cell adhesion, and to inhibit HIV replication. Brand et al, WO 98/35691, teaches that proteasome inhibitors, including boronic acid compounds, are useful for treating infaracts such as occur during stroke or myocardial infarction. Elliott et al, WO 99/15183, teaches that proteasome inhibitors are useful for treating inflammatory and autoimmune diseases.

Unfortunately, organoboronic acids can be relatively difficult to obtain in analytically pure form. For example, Snyder et al, *J. Am. Chem Soc.* 80: 3611 (1958), teaches that arylboronic acid compounds readily form cyclic trimeric anhydrides under dehydrating conditions. Also, alkylboronic acids and their boroxines are often air-sensitive. Korcek et al, *J. Chem. Soc. Perkin Trans.* 2 242 (1972), teaches that butylboronic acid is readily oxidized by air to generate 1-butanol and boric acid. Further, it has been found that the boropeptide TRI50c (discussed later in this application) and certain derivatives thereof tend to suffer degradation, resulting in an undesirable impurity level when the compounds are stored under normal conditions. These difficulties limit the pharmaceutical utility of boronic acid compounds, complicating the characterisation of pharmaceutical agents comprising boronic acid compounds and limiting their shelf life.

WO 02/059131 claims boronic acid products which are described as stable. In particular, these products are certain boropeptides and/or boropeptidomimetics in which the boronic acid group has been derivatised with a sugar. The claimed sugar derivatives, which have hydrophobic amino acid side chains, are of the formula



wherein:

P is hydrogen or an amino-group protecting moiety;

R is hydrogen or alkyl;

A is 0, 1 or 2;

R^1, R^2 and R^3 are independently hydrogen, alkyl, cycloalkyl, aryl or $-CH_2-R^5$;

R^5, in each instance, is one of aryl, aralkyl, alkaryl, cycloalkyl, heterocyclyl, heteroaryl, or $-W-R^6$, where W is a chalcogen and R^6 is alkyl;

where the ring portion of any of said aryl, aralkyl, alkaryl, cycloalkyl, heterocyclyl, or heteroaryl in R^1, R^2, R^3 or R^5 can be optionally substituted; and

Z^1 and Z^2 together form a moiety derived from a sugar, wherein the atom attached to boron in each case is an oxygen atom.

Some of the claimed compounds are sugar derivatives of the compound N-(2-pyrazine) carbonyl-phenylalanine-leucine boronic acid (LDP-341), an anti-cancer agent.

Many drugs comprise an active moiety which is a carboxylic acid. There are a number of differences between carboxylic acids and boronic acids, whose effects on drug delivery, stability and transport (amongst others) have not been investigated. One feature of trivalent boron compounds is that the boron atom is *sp*[2] hybridised, which leaves an empty $2p_z$ orbital on the boron atom. A molecule of the type BX_3 can therefore act as an electron-pair acceptor, or Lewis acid. It can use the empty $2p_z$ orbital to pick up a pair of nonbonding electrons from a Lewis base to form a covalent bond. BF_3 therefore reacts with Lewis bases such as NH_3 to form acid-base complexes in which all of the atoms have a filled shell of valence electrons.

Boric acid, accordingly, can act as a Lewis acid, accepting OH^-:

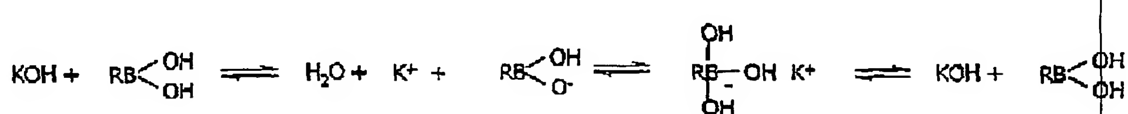
$$B(OH)_3 + H_2O \rightarrow B(OH)_4^- + H^+$$

Further, boronic acids of the type $RB(OH)_2$ are dibasic and have two pKa's. Another point of distinction about boron compounds is the unusually short length of bonds to boron, for which three factors may be responsible:

1. Formation of pπ- pπ bonds;
2. Ionic-covalent resonance;
3. Reduced repulsions between non-bonding electrons.

As previously mentioned, boronic acids can form cyclic trimeric anhydrides known as boroxines and the occurrence of boroxine is to be feared as it will potentially interfere with drug function.

The presumed equilibria of boronic and carboxylic acids in aqueous KOH are shown below (excluding formation of RBO_2^{2-}):



$$KOH + RCOOH \rightleftharpoons H_2O + K^+ + RCOO^-$$

Thrombosis

Hemostasis is the normal physiological process in which bleeding from an injured blood vessel is arrested. It is a dynamic and complex process in which proteolytic enzymes such as thrombin play a key role. Blood coagulation may occur through either of two cascades of zymogen activations, the extrinsic and intrinsic pathways of the coagulation cascade. Factor VIIa in the extrinsic pathway, and Factor IXa in the intrinsic pathway are important determinants of the activation of factor X to factor Xa, which itself catalyzes the activation of prothrombin to thrombin. The last protease in each pathway is thrombin, which acts to hydrolyze four small peptides (two FpA and two FpB) from each molecule of fibrinogen, thus deprotecting its polymerization sites. Once formed, the linear fibrin polymers may be cross-linked by factor XIIIa, which is itself activated by thrombin. In addition, thrombin is a potent activator of platelets, upon which it acts at specific receptors. Thrombin activation of platelets leads to aggregation of the cells and secretion of additional factors that further accelerate the creation of a hemostatic plug. Thrombin also potentiates its own production by the activation of factors V and VIII (see Hemker and Beguin in: Jolles, et. al., "Biology and Pathology of Platelet Vessel Wall Interactions," pp. 219-26 (1986), Crawford and Scrutton in: Bloom and Thomas, "Haemostasis and Thrombosis," pp. 47-77, (1987), Bevers, et. al., *Eur. J. Biochem.* **1982**, 122, 429-36, Mann, *Trends Biochem. Sci.* **1987**, 12, 229-33).

Proteases are enzymes which cleave proteins at specific peptide bonds. Cuypers et al., *J. Biol. Chem.* 257:7086 (1982), and the references cited therein, classify proteases on a mechanistic basis into five classes: serine, cysteinyl or thiol, acid or aspartyl, threonine and metalloproteases. Members of each class catalyse the hydrolysis of peptide bonds by a similar mechanism, have similar active site amino acid residues and are susceptible to class-specific inhibitors. For example, all serine proteases that have been characterised have an active site serine residue.

The coagulation proteases thrombin, factor Xa, factor VIIa, and factor IXa are serine proteases having trypsin-like specificity for the cleavage of sequence-specific Arg-Xxx peptide bonds. As with other serine proteases, the cleavage event begins with an attack of the active site serine on the scissile bond of the substrate, resulting in the formation of a tetrahedral intermediate. This is followed by collapse of the tetrahedral intermediate to form an acyl enzyme and release of the amino terminus of the cleaved sequence. Hydrolysis of the acyl enzyme then releases the carboxy terminus.

As indicated above, platelets play two important roles in normal hemostasis. First, by aggregating, they constitute the initial hemostatic plug which immediately curtails bleeding from broken blood vessels. Secondly, the platelet surface can become activated and potentiate blood clotting, a property referred to as platelet procoagulant activity. This may be observed as an increase in the rate of activation of prothrombin by factor Xa in the presence of factor Va and Ca^{2+}, referred to as the prothrombinase reaction. Normally, there are few (if any) clotting factors on the surface of unstimulated platelets but, when platelets are activated, negatively charged phospholipids (phosphatidylserine and phospatidylinositol) that are normally on the cytoplasmic side of the membrane become available and provide a surface on which two steps of the coagulation sequence occur. The phospholipid on the surface of activated platelets profoundly accelerates the reactions leading to the formation of thrombin, so that thrombin can be generated at a rate faster than its neutralisation by antithrombin III. The reactions that occur on the platelet surfaces are not easily inhibited by the natural anticoagulants in blood such as antithrombin III, either with or without heparin. (See Kelton and Hirsch in : Bloom and Thomas, "Haemostasis and Thrombosis," pp. 737-760, (1981); Mustard et al in : Bloom and Thomas, "Haemostasis and Thrombosis," pp. 503526, (1981); Goodwin et al; *Biochem. J.* **1995**, *308*, 15-21).

A thrombus can be considered as an abnormal product of a normal mechanism and can be defined as a mass or deposit formed from blood constituents on a surface of the cardiovascular system, for example of the heart or a blood vessel. Thrombosis can be regarded as the pathological condition wherein improper activity of the hemostatic mechanism results in intravascular thrombus formation. Three basic types of thrombi are recognised:

- the white thrombus which is usually seen in arteries and consists chiefly of platelets;
- the red thrombus which is found in veins and is composed predominantly of fibrin and red cells;
- the mixed thrombus which is composed of components of both white and red thrombi.

The composition of thrombi is influenced by the velocity of blood flow at their sites of formation. In general white platelet-rich thrombi form in high flow systems, while red coagulation thrombi form in regions of stasis. The high shear rate in arteries prevents the accumulation of coagulation intermediates on the arterial side of the circulation: only platelets have the capacity to form thrombi binding to the area of damage via von Willebrand factor. Such thrombi composed only of platelets are not stable and disperse. If the stimulus is strong then the thrombi will form again and then disperse continually until the stimulus has diminished. For the thrombus to stabilise, fibrin must form. In this respect, small amounts of thrombin can accumulate within the platelet thrombus and activate factor Va and stimulate the platelet procoagulant activity. These two events lead to an overall increase in the rate of activation of prothrombin by factor Xa of 300,000 fold. Fibrin deposition stabilises the platelet thrombus.

Thrombin inhibitors are not clinically effective at inhibiting stimulation of platelet procoagulant activity. Accordingly, a therapeutic agent which inhibits platelet procoagulant activity would be useful for treating or preventing arterial thrombotic conditions

On the venous side of circulation, the thrombus is comprised of fibrin: thrombin can accumulate because of the slower flow on the venous side and platelets play only a minor role.

Thrombosis is thus not considered to be a single indication but, rather, is a class of indications embracing distinct sub-classes for which differing therapeutic agents and/or protocols may be appropriate. Thus, regulatory authorities treat disorders such as, for example, deep vein thrombosis, cerebrovascular arterial thrombosis and pulmonary embolism as distinct indications for the purposes of licensing medicines. Two main sub-classes of thrombosis are arterial thrombosis and venous thrombosis. Arterial thrombosis includes such specific disorders as acute coronary syndromes [for example acute myocardial infarction (heart attack, caused by thrombosis in a coronary artery)], cerebrovascular arterial thrombosis (stroke, caused by thrombosis in the cerebrovascular arterial system) and peripheral arterial thrombosis. Examples of conditions caused by venous thrombosis are deep vein thrombosis and pulmonary embolism.

The management of thrombosis commonly involves the use of thrombolytic agents in combination with anticoagulants and antiplatelet drugs (inhibitors of platelet aggregation) to lyse the newly formed clot and to control future thrombogenesis. Anticoagulants are used also in the treatment of patients thought susceptible to thrombosis.

Currently, two of the most effective classes of drugs in clinical use as anticoagulants are the heparins and the vitamin K antagonists. The heparins are ill-defined mixtures of sulfated polysaccharides that bind to, and thus potentiate the action of antithrombin III. Antithrombin III is a naturally occurring inhibitor of the activated clotting factors IXa, Xa, XIa, thrombin and probably XIIa (see Jaques, *Pharmacol. Rev.* **1980**, 31, pp. 99-166). The vitamin K antagonists, of which warfarin is the most well-known example, act indirectly by inhibiting the post-ribosomal carboxylations of the vitamin K dependent coagulation factors II, VII, IX and X (see Hirsch, *Semin. Thromb. Hemostasis* **1986**, 12, 1-11). While effective therapies for the treatment of thrombosis, heparins and vitamin K antagonists have the unfortunate side effects of bleeding, heparin-induced thrombocytopenia (in the case of heparin) and marked interpatient variability, resulting in a small and unpredictable therapeutic safety margin.

The use of direct acting inhibitors of thrombin and other serine protease enzymes of the coagulation system is expected to alleviate these problems. To that end, a wide variety of serine protease inhibitors have been tested, including boropeptides, i.e. peptides containing a boronic acid analogue of an N-acyl-α-amino acid. Whilst direct acting boronic acid thrombin inhibitors

have been discussed earlier in this specification, they are further described in the following paragraph.

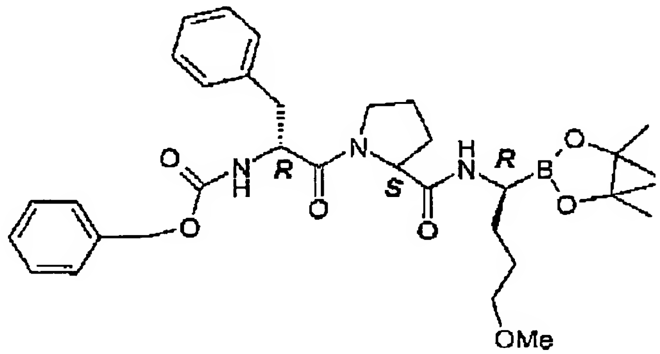
Neutral P1 Residue Boropeptide Thrombin Inhibitors

Claeson et al (US 5574014 and others) and Kakkar et al (WO 92/07869 and family members including US 5648338) disclose lipophilic thrombin inhibitors having a neutral (uncharged) C-terminal side chain, for example an alkoxyalkyl side chain. The aforementioned US patents of Claeson et al and Kakkar et al (US 5574014 and US 5648338) are incorporated herein by reference.

The Claeson et al and Kakkar et al patent families disclose boronate esters containing the amino acid sequence D-Phe-Pro-BoroMpg [(R)-Phe-Pro-BoroMpg], which are highly specific inhibitors of thrombin. Of these compounds may be mentioned in particular Cbz-(R)-Phe-Pro-BoroMpg-OPinacol (also known as TRI50b). The corresponding free boronic acid is known as TRI50c. For further information relating to TRI50b and related compounds, the reader is referred to the following documents, all incorporated herein by reference:

- Elgendy S et al., in *The Design of Synthetic Inhibitors of Thrombin*, Claeson G et al Eds, *Advances in Experimental Medicine*, **1993**, *340*, pp, pp 173-178.
- Claeson G et al, *Biochem J.* **1993**, *290*, 309-312
- Tapparelli C et al, *J Biol Chem*, **1993**, *268*, 4734-4741
- Claeson G, in *The Design of Synthetic Inhibitors of Thrombin*, Claeson G et al Eds, *Advances in Experimental Medicine*, **1993**, *340*, pp 83-91
- Phillip et al, in *The Design of Synthetic Inhibitors of Thrombin*, Claeson G et al Eds, *Advances in Experimental Medicine*, **1993**, *340*, pp 67-77
- Tapparelli C et al, *Trends Pharmacol. Sci.* **1993**, *14*, 366-376
- Claeson G, *Blood Coagulation and Fibrinolysis* **1994**, *5*, 411-436
- Elgendy et al, *Tetrahedron* **1994**, *50*, 3803-3812
- Deadman J et al, *J. Enzyme Inhibition* **1995**, *9*, 29-41.
- Deadman J et al, *J. Medicinal Chemistry* **1995**, *38*, 1511-1522.

The tripeptide sequence of TRI50b has three chiral centres. The Phe residue is considered to be of R (= D) configuration and the Pro residue of natural S (= L) configuration, at least in compounds with commercially useful inhibitor activity; the Mpg residue is believed to be of R (= L) configuration in isomers with commercially useful inhibitor activity. Thus, the most effective TRI50b stereoisomer is considered to be of RSR configuration and may be represented as:



(RSR)-TRI50b: Cbz-(R)-Phe-(S)-Pro-(R)-boroMpg Pinacol

Whilst direct acting thrombin inhibitors have been found useful for the treatment of patients susceptible to or suffering from venous thrombosis, the same is not true of arterial thrombosis. In the case of currently available thrombin inhibitors, it would be necessary to raise the dosage used in the treatment of venous thrombosis by many times in order to treat (prevent) arterial thrombosis. Such raised dosages typically cause bleeding, which makes direct acting thrombin inhibitors unsuitable for treating arterial thrombosis. Heparin, which primarily acts as a thrombin inhibitor, is also unsuitable to treat arterial thrombosis. It has been found that a class of compounds which is defined by Formula III below and represented by boropeptides having the amino acid sequence (R)-Phe-Pro-BoroMpg is beneficial in that the members of the class are useful for treating arterial thrombosis by therapy or prophylaxis.

Oral Absorption

Absorption in the gastro-intestinal tract can be by an active or a passive route. Active absorption by transport mechanisms tends to be variable between individuals and with intestinal content (Gustaffson, D Thromb.Res., 2001, 101, 171-181). The upper intestine has been identified as the principal site of oral drug absorption. In particular, the duodenum is the customary target site for absorption of orally administered drugs because of its large surface area. The intestinal mucosa acts as a barrier that controls passive transcellular absorption: the absorption of ionic species is blocked whilst the transcellular absorption of lipophilic molecules is favoured (Palm K et al., J.Pharmacol and Exp. Therapeutics, 1999, 291,435-443).

Orally administered drugs are required to be consistently and adequately absorbed. Variability of absorption between individuals or between different occasions in the same individual is unwelcome. Similarly, drugs which have a low level of bioavailability (only a small portion of the administered active agent is absorbed) are generally unacceptable.

Non-ionised compounds are favoured for passive absorption, a route associated with invariability, and are therefore preferred for consistent absorption. Lipophilic species are particularly favoured by passive absorption mechanisms and, accordingly, non-ionic, lipophilic drugs are indicated to be most favoured for consistent and high oral absorption.

Many organoboronic acid compounds may be classified as lipophilic or hydrophobic. Typically, such compounds include amongst others:

- boropeptides of which all or a majority of the amino acids are hydrophobic
- boropeptides of which at least half of the amino acids are hydrophobic and which have a hydrophobic N-terminal substituent
- non-peptides based on hydrophobic moieties.

Hydrophobic amino acids include those whose side chain is hydrocarbyl, hydrocarbyl containing an in-chain oxygen and/or linked to the remainder of the molecule by an in-chain oxygen or heteroaryl, or any of the aforesaid groups when substituted by hydroxy, halogen or trifluoromethyl. Representative hydrophobic side chains include alkyl, alkoxyalkyl, either of the aforesaid when substituted by at least one aryl or heteroaryl, aryl, heteroaryl, aryl substituted by at least one alkyl and heteroaryl substituted by at least one alkyl. Proline and other imino acids which are ring-substituted by nothing or by one of the moieties listed in the previous sentence are also hydrophobic.

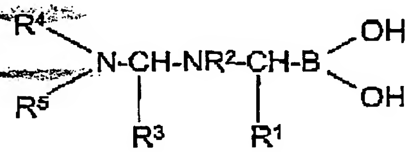
Some hydrophobic side chains contain from 1 to 20 carbon atoms, e.g. non-cyclic moieties having 1, 2, 3 or 4 carbon atoms. Side chains comprising a cyclic group typically but not necessarily contain from 5 to 13 ring members and in many cases are phenyl or alkyl substituted by one or two phenyls.

Hydrophobic non-peptides are typically based on moieties which may form a side chain of a hydrophobic amino acid, as described above.

Hydrophobic compounds may contain, for example, one amino group and/or one acid group (e.g. -COOH, $-B(OH)_2$). Generally, they do not contain multiple polar groups of any one type.

One class of hydrophobic organoboronic acids have a partition coefficient between 1-n-octanol and water expressed as log P of greater than 1 at physiological pH and 25°C. For example, TRI50c has a partition coefficient of approximately 2.

A sub-class of hydrophobic organoboronic acids (which sub-class includes both TRI50c and LDP341) is of the formula (I):



(I)

where:

R^1 is H or a neutral side group;

R^2 is H or C_1-C_{13} hydrocarbyl optionally containing in-chain oxygen or sulfur and optionally substituted by a substituent selected from halo, hydroxy and trifluoromethyl;

or R^1 and R^2 together form a C_1-C_{13} moiety which in combination with N-CH forms a 4-6 membered ring and which is selected from alkylene (whether branched or linear) and alkylene containing an in-chain sulfur or linked to N-CH through a sulfur;

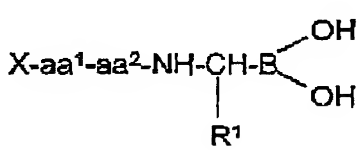
R^3 is the same as or different from R^1 provided that no more than one of R^1 and R^2 is H, and is H or a neutral side group;

R^4 is H or C_1-C_{13} hydrocarbyl optionally containing in-chain oxygen or sulfur and optionally substituted by a substituent selected from halo, hydroxy and trifluoromethyl;

or R^3 and R^4 together form a C_1-C_{13} moiety which in combination with N-CH forms a 4-6 membered ring and which is selected from alkylene (whether branched or linear) and alkylene containing an in-chain sulfur or linked to N-CH through a sulfur; and

R^5 is X-E- wherein E is nothing or a hydrophobic moiety selected from the group consisting of amino acids (natural or unnatural) and peptides of two or more amino acids (natural or unnatural) of which more than half are hydrophobic and X is H or an amino-protecting group.

A more preferred sub-set of hydrophobic compounds, which includes TRI50c, comprises peptide boronic acids of formula (III):



(III)

where:

X is H (to form NH_2) or an amino-protecting group;

aa^1 is Phe, Dpa or a wholly or partially hydrogenated analogue thereof;

aa^2 is an imino acid having from 4 to 6 ring members; and

R^1 is a group of the formula $-(CH_2)_m-W$, where m is 2, 3 or 4 and W is -OH, -OMe, -OEt or halogen (F, Cl, Br or I).

Typical functionalities required for interaction of drugs with their physiological targets are functional groups such as carboxylic and sulphonic acids. These groups exist as the protonated form in the stomach (at pH 2-3), but will be ionised to some extent at the higher pH of the intestinal fluid. One strategy that has been used to avoid the ionisation of the carboxylates or sulphonates is to present them as ester forms, which are cleaved once absorbed into the vascular lumen.

For example, the direct acting thrombin inhibitor Melagatran, which has sub-optimal gastrointestinal absorption, has terminal carboxy and amidino groups and is a pure zwitterion at pH 8-10 when the carboxylic acid and amidino groups are both charged. A prodrug H 376/95 was therefore developed which has protecting groups for the carboxylic acid and for the amidine and is a more lipophilic molecule than Melagatran. The prodrug has a permeability coefficient across cultured epithelial Caco-2 cells 80 times higher than that of Melagatran and oral bioavailability 2.7-5.5 times higher than that of Melagatran as well as much smaller variability in the area under the drug plasma concentration vs. time curve (Gustafsson et al, *Thrombosis Research* 101: 171-181 (2001)).

Oral Absorption of Boropeptides, Boropeptidomimetics and other Organoboronates

The boronate ester group of TRI50b is rapidly cleaved in the conditions of the plasma to form the corresponding boronic acid group, which is considered to be the active moiety which inhibits the catalytic site of thrombin.

Boronic acids are divalent functional groups, with boron-oxygen bond lengths (1.6Å) more typical of single bonds, unlike superficially comparable C-O and S-O bonds in carboxylic and sulphonic acids. Consequently the boronic acid group has two ionisation potentials. The boronic acid group will be partly ionised at pH's of the duodenal fluid and not suited to the desired passive duodenal uptake. Thus, a charged boronate inhibitor H-D-PheProBoroArg is absorbed by a predominantly active transport mechanism (Saitoh, H. and Aungst, B.J., Pharm. Res., 1999, 16, 1786-1789).

The peptide boronic acid formed by such cleavage of TRI50b is relatively insoluble in water, especially at acidic or neutral pH, and tends to be poorly absorbed in the stomach and duodenum. The acid has the structure Cbz-Phe-Pro-BoroMpg-OH.

Whereas the peptide boronic acid Cbz-Phe-Pro-BoroMpg-OH is partly ionised under duodenal conditions and, to that extent, unfavoured for passive transport, esters of the acid are designed for a high rate of passive (thus consistent) transport. The tripeptide sequence Phe-Pro-Mpg belongs to an unusual class of serine protease inhibitory peptide sequences in having a non-basic P1 side chain (specifically, methoxypropyl), such that the tripeptide consists of three non-polar amino acids. The esters of the peptide boronic acid are non-ionisable and the ester-forming species further impart lipophilic properties, so encouraging a high rate of passive transport.

Computational techniques have confirmed that TRI50b and other diol esters of Cbz-Phe-Pro-BoroMpg-OH can be predicted to have good bioavailability. Thus, polar surface area (PSAd) is a parameter predictive of bioavailability and PSAd values of greater than 60Å correlate well with passive transcellular transport and with bioavailability of known drugs (Kelder, J. Pharm. Res., 1999, 16, 1514-1519). Measurements for diol esters of the above peptide boronic acid, including the pinacol ester TRI50b, show that the diol esters have PSAd values well above 60Å, predictive of passive transport and good bioavailability as shown in Table 1:

Table 1: PSAd values of selected diol esters of Cbz-Phe-Pro-BoroMpg-OH

Diol	PSAd Value
Pinacol	98.74
Pinanediol	90.64

The corresponding monohydroxy alcohol (e.g. alkoxy) esters were considered too unstable, spontaneously cleaving to liberate the acid *in-vitro*. Esters of diols such as pinanediol and pinacol have enhanced kinetic stability over esters of monohydroxy alcohols, in that after partial hydrolysis to the mono-ester derivative they will tend to reassociate by a facile intra-molecular reaction.

To counterbalance these highly desirable features of TRI50b, it has been discovered that TRI50b tends to hydrolyse in acid media. Thus in the acid conditions of an HPLC assay, TRI50b is converted to the acid form with a short half life, which implies potential intraduodenal hydrolysis into ionic species which would resist passive transport and, if anything, be absorbed by active transport, indicative at best of variable bioavailability.

The instability of TRI50b to hydrolysis also presents potential disadvantages in preparation of the compound and its formulation, as well as in the storage of pharmaceutical formulations containing it.

A more challenging difficulty which has been posed by TRI50b is that the data show significant variation in bioavailability between subjects. Such variability can make a drug candidate unacceptable and it would therefore be desirable to reduce the observed variability.

An ideal solution to the instability of TRI50b would be development of a diol ester more stable to hydrolysis. In this regard, it is known that ring size can affect boronate stability and glycolato boron has been shown to have enhanced aqueous stability compared to pinacol (D.S.Matteson, Stereodirected Synthesis with Organoboranes, Springer-Verlag, 1995, ch.1). Similarly, the pinanediol ester is more stable than the pinacol; this is believed to be because the pinanediol group is highly sterically hindered and disfavours nucleophilic attack on the boron. In fact transesterification from pinacol to pinanediol has been reported (Brosz, CS, Tet. Assym, 1997, 8, 1435-1440) whereas the reverse process is unfavourable. The pinanediol ester however is considered too slow to cleave in plasma and there remains a need to provide an improved diol ester.

Another solution to the instability of TRI50b would be to administer in its place TRI50c. However, TRI50c data suggest that TRI50c too suffers from variability in bioavailability. The reasons for such apparent variability of TRI50b and TRI50c are not known and it has therefore not been possible to propose a rational solution to the problem.

The properties described above will be shared by similar hydrophobic, non-basic boropeptides.

The present invention is predicated on the finding that certain organoboronic acid products provide unexpectedly favourable bioavailability. The products are further indicated to be of enhanced stability.

The benefits of the present invention include a solution to the problem of boronate diol ester and especially TRI50b instability and variability, that is to say the products of the invention provide compounds which are more stable than TRI50b and other comparable esters and which have lower variability in bioavailability.

BRIEF SUMMARY OF THE INVENTION

In one aspect, the invention provides a salt of a pharmaceutically acceptable multivalent (at least divalent) metal and an organoboronic acid drug. Such salts are not only contrary to the direction

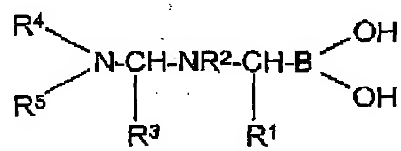
of the prior art but additionally have unexpectedly high and consistent oral bioavailability not susceptible of explanation on the basis of known mechanisms.

The invention includes a class of salts in which the drug has no charged group at physiological pH other than its boronate (boronic acid) moiety.

In preferred embodiments the organoboronic acid is hydrophobic. Preferred organoboronic acids have a partition coefficient between 1-n-octanol and water expressed as log P of greater than 1.0 at physiological pH and 25°C.

One preferred class of salts comprises those wherein the organoboronic acid comprises a boropeptide or boropeptidomimetic. Boropeptide drugs which may beneficially be prepared as salts of the invention include without limitation those of the formula X-$(aa)_n$-$B(OH)_2$, where X is H or an amino-protecting group, n is 2, 3 or 4, (especially 2 or 3) and each aa is independently a hydrophobic amino acid, whether natural or unnatural.

In a sub-class of the salts of boropeptides/boropeptidomimetics, the organoboronic acid is of the formula (I):



(I)

where:

R^1 is H or a neutral side group;

R^2 is H or C_1-C_{13} hydrocarbyl optionally containing in-chain oxygen or sulfur and optionally substituted by a substituent selected from halo, hydroxy and trifluoromethyl;

or R^1 and R^2 together form a C_1-C_{13} moiety which in combination with N-CH forms a 4-6 membered ring and which is selected from alkylene (whether branched or linear) and alkylene containing an in-chain sulfur or linked to N-CH through a sulfur;

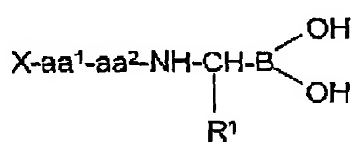
R^3 is the same as or different from R^1 provided that no more than one of R^1 and R^2 is H, and is H or a neutral side group;

R^4 is H or C_1-C_{13} hydrocarbyl optionally containing in-chain oxygen or sulfur and optionally substituted by a substituent selected from halo, hydroxy and trifluoromethyl;

or R^3 and R^4 together form a C_1-C_{13} moiety which in combination with N-CH forms a 4-6 membered ring and which is selected from alkylene (whether branched or linear) and alkylene containing an in-chain sulfur or linked to N-CH through a sulfur; and

R^5 is X-E- wherein E is nothing or a hydrophobic moiety selected from the group consisting of amino acids (natural or unnatural) and peptides of two or more amino acids (natural or unnatural) of which more than half are hydrophobic and X is H or an amino-protecting group.

The present invention includes pharmaceutically acceptable multivalent metal salts of a peptide boronic acid of formula (III):



(III)

where:

X is H (to form NH_2) or an amino-protecting group;

aa^1 is Phe, Dpa or a wholly or partially hydrogenated analogue thereof;

aa^2 is an imino acid having from 4 to 6 ring members; and

R^1 is a group of the formula $-(CH_2)_m-W$, where m is 2, 3 or 4 and W is $-OH$, $-OMe$, $-OEt$ or halogen (F, Cl, Br or I).

The boronic acids of formula (III) inhibit thrombin. They exhibit anti-thrombotic activity in both venous and arterial contexts, and are considered to inhibit platelet pro-coagulant activity. The most preferred boronic acid of formula (III) is TRI50c.

The Examples of this patent application contain data showing that the calcium salt of TRI50c is markedly less soluble than the potassium salt and yet has higher oral bioavailability and higher consistency of oral bioavailability. The finding of an inverse relationship between solubility and bioavailability of two salts is particularly unpredictable. There is no known property of organoboronic acid drugs which accounts for this finding. The invention therefore includes

amongst other subject matter a TRI50c derivative which enhances stability as compared with TRI50b and reduces the variability in absorption which has been observed with TRI50b and TRI50c, and advantageously enables adequately consistent and high bioavailability.

The family of compounds represented by formula (III) represents near neighbours of TRI50c.

TRI50c is distinguished from most other organic acid drugs in that the acid group of TRI50c is a boronic acid and not a carboxylic acid. The data in this application are indicative of multivalent metal salts of organoboronic acid drugs providing a technical effect, not linked to solubility, which enhances the amount and consistency of bioavailability. It does not follow that, because the effect is not linked to solubility, there will in every individual case be for that acid a quantitative relationship between solubility and bioavailability like that observed for TRI50c.

There is a debate in the literature as to whether boronates in aqueous solution form the 'trigonal' $B(OH)_2$ or 'tetrahedral' $B(OH)_3^-$ boron species, but NMR evidence seems to indicate that at a pH below the first pKa of the boronic acid the main boron species is the neutral $B(OH)_2$. In the duodenum the pH is likely to be between 6 and 7, so the trigonal species is likely to be predominant here. In any event, the symbol $-B(OH)_2$ includes tetrahedral as well as trigonal boron species.

The invention includes also oral formulations of the salts of the invention.

According to a further aspect of the present invention, there is provided a method of treatment of a condition where anti-thrombotic activity is required which method comprises oral administration of a therapeutically effective amount of a multivalent metal salt of a boronic acid of formula III to a person suffering from, or at risk of suffering from, such a condition.

The salts of the invention include products obtainable by (have the characteristics of a product obtained by) reaction of the boronic acid with a base of a multivalent metal and the term "salt" herein is to be understood accordingly. The term "salt" in relation to the products of the invention, therefore, does not necessarily imply that the products contain discrete cations and anions and is to be understood as embracing products which are obtainable using a reaction of a boronic acid and a base. The invention embraces products which, to a greater or lesser extent, are in the form of a coordination compound. The invention thus provides also products obtainable by (having the characteristics of a product obtained by) reaction of an organoboronic acid drug with a multivalent metal base a well as the therapeutic, including prophylactic, use of such products.

The invention is not limited as to the method of preparation of the salts, provided that they contain a multivalent metal and a pharmaceutically useful organoboronate species. It is not required that the salts be prepared by reaction of a base of the multivalent metal and the organoboronic acid drug. Further, the invention includes salts indirectly prepared by such an acid/base reaction as well as salts obtainable by (having the characteristics of a products obtained by) such indirect preparation. As examples of indirect preparation may be mentioned processes in which, after initial recovery of the salt, it is purified and/or treated to modify its physicochemical properties, for example to modify solid form (e.g. crystal form) or hydrate form, or both.

The salts may be in isolated form. The salts may have a purity of at least 90%, e.g. of greater than or equal to 95%, for example purities of up to 99.5%. In the case of pharmaceutical formulations, such salt forms may be combined with pharmaceutically acceptable diluents, excipients or carriers.

The invention includes a method for preparing the salts from the corresponding boronic acid as an intermediate, as well as the intermediate boronic acid of Formula III and a method for preparing it.

Further aspects and embodiments of the invention are set forth in the following description and claims.

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of the words, for example "comprising" and "comprises", mean "including but not limited to", and are not intended to (and do not) exclude other moieties, additives, components, integers or steps.

DETAILED DESCRIPTION OF THE INVENTION

Glossary

The following terms and abbreviations are used in this specification:

α-Aminoboronic acid or Boro(aa) refers to an amino acid in which the CO_2 group has been replaced by BO_2

The term "amino-group protecting moiety" refers to any group used to derivatise an amino group, especially an N-terminal amino group of a peptide or amino acid. Such groups include,

without limitation, alkyl, acyl, alkoxycarbonyl, aminocarbonyl, and sulfonyl moieties. However, the term "amino-group protecting moiety" is not intended to be limited to those particular protecting groups that are commonly employed in organic synthesis, nor is it intended to be limited to groups that are readily cleavable.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings or animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The term "heteroaryl" refers to a ring system which has at least one (e.g. 1, 2 or 3) in-ring heteroatoms and has a conjugated in-ring double bond system. The term "heteroatom" includes oxygen, sulfur and nitrogen, of which sulfur is sometimes less preferred.

"Natural amino acid" means an L-amino acid (or residue thereof) selected from the following group of neutral (hydrophobic or polar), positively charged and negatively charged amino acids:

Hydrophobic amino acids
A = Ala = alanine
V = Val = valine
I = Ile = isoleucine
L = Leu = leucine
M = Met = methionine
F = Phe = phenylalanine
P = Pro = proline
W = Trp = tryptophan

Polar (neutral or uncharged) amino acids
N = Asn = asparagine
C = Cys = cysteine
Q = Gln = glutamine
G = Gly = glycine
S = Ser = serine
T = Thr = threonine
Y = Tyr = tyrosine

Positively charged (basic) amino acids
R = Arg = arginine

H = His = histidine

K = Lys = lysine

Negatively charged amino acids

D = Asp = aspartic acid

E = Glu = glutamic acid.

Cbz – benzyloxycarbonyl

Cha – cyclohexylalanine (a hydrophobic unnatural amino acid)

Charged - carrying a charge at physiological pH, as in the case of an amino, amidino or carboxy group

Dcha – dicyclohexylalanine (a hydrophobic unnatural amino acid)

Dpa – diphenylalanine (a hydrophobic unnatural amino acid)

Drug - a pharmaceutically useful substance, whether the active in vivo principle or a prodrug

Mpg – 3-methoxypropylglycyl (a hydrophobic unnatural amino acid)

Multivalent – valency of at least two, for example two or three

Pinac = Pinacol – 2,3-dimethyl-2,3-butanediol

(+)-Pinanediol boronate – 1a,7,7-trimethyl-[1aS-(1aa, 4a, 6a, 5aa)]-4,6-methano-1,2-benzodioxaborole

pNA - p-nitroanilide

Pip - pipecolinic acid

THF - tetrahydrofuran

Thr – thrombin

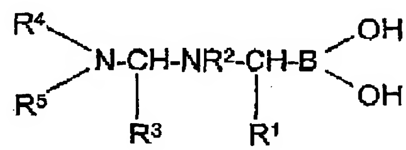
The Compounds

The products of the invention comprise a salt of a pharmaceutically acceptable multivalent (at least divalent) metal and an organoboronic acid drug (where the term "drug" embraces prodrugs). As previously stated, the term "salt" refers to a product containing a multivalent metal and an organoboronate species, for example a product having the characteristics of a product of a reaction between such an acid and a base comprising a multivalent metal (for example a +2 ion); in particular, such characteristics comprise the identity of the multivalent metal and of the drug species.

The acid may for example be any boronic acid mentioned under the heading "BACKGROUND OF THE INVENTION" or in any document referred to under that heading, e.g. TRI50c or LDP-341.

In preferred embodiments the organoboronic acid is hydrophobic.

One preferred class of salts comprises those wherein the organoboronic acid comprises a boropeptide or boropeptidomimetic. For example, in a sub-class of these salts the organoboronic acid is of the formula (I):



(I)

where:

R^1 is H or a non-charged side group;

R^2 is H or C_1-C_{13} hydrocarbyl optionally containing in-chain oxygen or sulfur and optionally substituted by a substituent selected from halo, hydroxy and trifluoromethyl;

or R^1 and R^2 together form a C_1-C_{13} moiety which in combination with N-CH forms a 4-6 membered ring and which is selected from alkylene (whether branched or linear) and alkylene containing an in-chain sulfur or linked to N-CH through a sulfur;

R^3 is the same as or different from R^1 provided that no more than one of R^1 and R^2 is H, and is H or a non-charged side group;

R^4 is H or C_1-C_{13} hydrocarbyl optionally containing in-chain oxygen or sulfur and optionally substituted by a substituent selected from halo, hydroxy and trifluoromethyl;

or R^3 and R^4 together form a C_1-C_{13} moiety which in combination with N-CH forms a 4-6 membered ring and which is selected from alkylene (whether branched or linear) and alkylene containing an in-chain sulfur or linked to N-CH through a sulfur; and

R^5 is X-E- wherein E is nothing or a hydrophobic moiety selected from the group consisting of amino acids (natural or unnatural) and peptides of two or more amino acids (natural or unnatural) of which more than half are hydrophobic and X is H or an amino-protecting group.

Preferably R^1 is non polar.

Preferably hydrocarbyl is selected from the group consisting of alkyl; alkyl substituted by cycloalkyl, aryl or heterocyclyl; cycloalkyl; aryl; and heterocyclyl. Heterocyclyl is preferably heteroaryl.

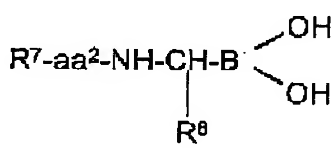
A preferred class of compounds have R^2 as H.

Preferably, R^4 is H or R^3 and R^4 together form a said C_1-C_{13} moiety.

In one class of compounds E is nothing.

In another class, E is a hydrophobic amino acid.

One preferred class of salts comprises those in which the organoboronic acid is of the formula (II):



(II)

wherein

R^7 is X-E'- wherein X is hydrogen or an amino-protecting group and E' is absent or is a hydrophobic amino acid;

R^8 is an optionally substituted moiety containing from 1 to 4 carbon atoms selected from the group consisting of alkyl, alkoxy and alkoxyalkyl, the optional substituents being hydroxy or, preferably, halogen (F, Cl, Br, I); and

aa^2 is a hydrophobic amino acid.

R^7 is preferably X-, or X-Phe or X-Dpa.

R^8 is preferably not substituted. R^8 is preferably a C_4 group, e.g. alkyl or alkoxyalkyl, such as 2-methylpropyl or 3-methoxypropyl, for example.

The hydrophobic amino acids may for example have a side chain which is hydrocarbyl or heteroaryl, or which includes both hydrocarbyl and heteroaryl residues. The hydrocarbyl residues optionally contain in-chain oxygen; they may be substituted by, for example, halogen or hydroxy

(but usually not more than one hydroxy group). Alternatively, hydrophobic amino acids may be proline or another imino acid.

aa^2 is preferably a natural hydrophobic amino acid, e.g. Pro or Phe.

Preferably X is R^6-$(CH_2)_p$-C(O)-, R^6-$(CH_2)_p$-$S(O)_2$-, R^6-$(CH_2)_p$-NH-C(O)- or R^6-$(CH_2)_p$-O-C(O)- wherein p is 0, 1, 2, 3, 4, 5 or 6 (of which 0 and 1 are preferred) and R^6 is H or a 5 to 13-membered cyclic group optionally substituted by 1, 2 or 3 substituents selected from halogen, amino, nitro, hydroxy, a C_5-C_6 cyclic group, C_1-C_4 alkyl and C_1-C_4 alkyl containing, and/or linked to the 5 to 13-membered cyclic group through, an in-chain O, the aforesaid alkyl groups optionally being substituted by a substituent selected from halogen, amino, nitro, hydroxy and a C_5-C_6 cyclic group. More preferably X is R^6-$(CH_2)_p$-C(O)- or R^6-$(CH_2)_p$-O-C(O)- and p is 0 or 1. Said 5 to 13-membered cyclic group is often aromatic or heteroaromatic, for example is a 6-membered aromatic or heteroaromatic group. In many cases, the group is not substituted.

Exemplary X groups are (2-pyrazine) carbonyl, (2-pyrazine) sulfonyl and benzyloxycarbonyl.

In a preferred class of boronic acids, which are anti-thrombotic and include TRI50c, the peptide boronic acid is of formula (III):

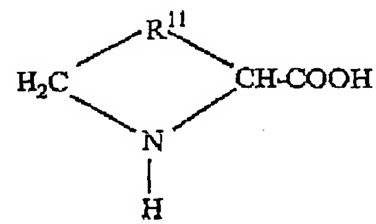
$$\text{X-aa}^1\text{-aa}^2\text{-NH-}\underset{\underset{\displaystyle R^9}{|}}{\text{CH}}\text{-B}\begin{matrix}\diagup \text{OH}\\ \diagdown \text{OH}\end{matrix} \qquad \text{(III)}$$

X is a moiety bonded to the N-terminal amino group and may be H to form NH_2. The identity of X is not critical to the invention but may be a preferred X moiety described above. As a preferred example there may be mentioned benzyloxycarbonyl.

aa^1 is Phe, Dpa or a wholly or partially hydrogenated analogue thereof. The wholly hydrogenated analogues are Cha and D-Dcha.

A preferred class of products comprises those in which aa^2 is a residue of an imino acid of formula (IV)

(IV),



where R^{11} is $-CH_2-$, CH_2-CH_2-, $-S-CH_2-$ or $-CH_2-CH_2-CH_2-$, which group when the ring is 5 or 6-membered is optionally substituted at one or more $-CH_2-$ groups by from 1 to 3 C_1-C_3 alkyl groups, for example to form the R^{11} group $-S-C(CH_3)_2-$. Of these imino acids, azetidine-2-carboxylic acid, especially (s)-azetidine-2-carboxylic acid, and more particularly proline are preferred.

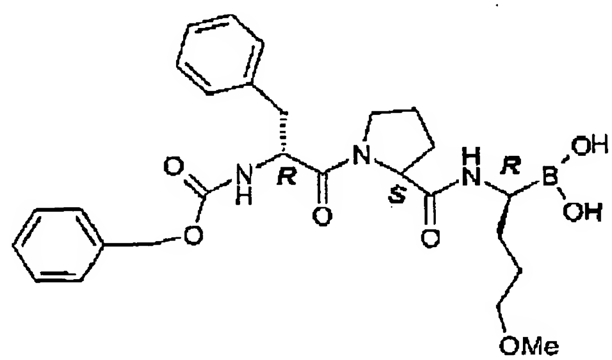
It will be appreciated from the above that a very preferred class of products consists of those in which aa^1-aa^2 is Phe-Pro. In another preferred class, aa^1-aa^2 is Dpa-Pro. In other products, aa^1-aa^2 is Cha-Pro or Dcha-Pro. Of course, the invention includes corresponding product classes in which Pro is replaced by (s)-azetidine-2-carboxylic acid.

R^9 is a group of the formula $-(CH_2)_m-W$. Integer m is 2, 3 or 4 and W is –OH, –OMe, –OEt or halogen (F, Cl, I or, preferably, Br). The most preferred W groups are –OMe and –OEt, especially –OMe. It is preferred that m is 3 for all W groups and, indeed, for all formula (III) compounds. Particularly preferred R^9 groups are 2-bromoethyl, 2-chloroethyl, 2-methoxyethyl, 4-bromobutyl, 4-chlorobutyl, 4-methoxybutyl and, especially, 3-bromopropyl, 3-chloropropyl and 3-methoxypropyl. Most preferably, R^9 is 3-methoxypropyl. 2-Ethoxyethyl is another preferred R^9 group.

Accordingly, a very preferred class of salts consists of those of acids of the formula X-Phe-Pro-Mpg-$B(OH)_2$, especially Cbz-Phe-Pro-Mpg-$B(OH)_2$; also preferred are analogues of these compounds in which Mpg is replaced by a residue with another of the particularly preferred R^9 groups and/or Phe is replaced by Dpa or another aa^1 residue.

The aa^1 moiety of the salts of the formula (III) acids is preferably of R configuration (D-configuration). The aa^2 moiety is preferably of S configuration (L-configuration). Particularly preferred salts have aa^1 of R configuration and aa^2 of S configuration. The chiral centre –NH-CH(R^9)-B- is preferably of R configuration. It is considered that commercial formulations will

have the chiral centres in RSR arrangement, as for example in the case of salts of Cbz-Phe-Pro-BoroMpg-OH:



Cbz-(R)-Phe-(S)-Pro-(R)-boroMpg-OH

The salts of the invention correspond to reaction products of an organoboronic acid as described above with a base of a multivalent metal, i.e. a metal having a valency of two or more. The metal is preferably:

1. a Group II metal (alkaline earth metal);

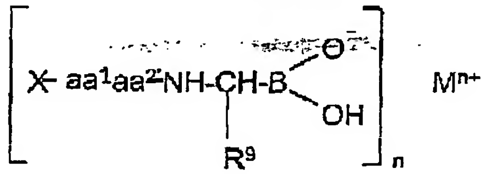
2. another pharmaceutically acceptable divalent metal, e.g. zinc;

3. a Group III metal.

One especially preferred class of salts comprises divalent metal salts. A particularly preferred class of salts comprises the calcium salts. Another particularly preferred class of salts comprises the magnesium salts. A further class of salts comprises the zinc salts.

Preferred salts are of the monovalent boronate though in practice the monovalent salts may contain a very small proportion of the divalent boronate. The term "monovalent boronate" refers to trigonal $-B(OH)_2$ groups in which one of the B-OH groups is deprotonated as well as to corresponding tetrahedral groups in equilibrium therewith.

The invention includes therefore products (compositions of matter) which comprise salts which may be represented by formula (V):



(V)

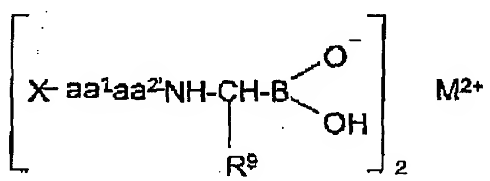
where M^{n+} is a divalent or trivalent metal cation, $aa^{2'}$ is a residue of an imino acid of formula IV, n is 2 or 3 as the case may be, and aa^1, X and R^9 are as defined above. As previously indicated, the boronate may comprise a tetrahedral species.

Considering the metals in turn:

<u>1. Divalent, e.g. alkaline earth metal (Group II metal) salts</u>

A preferred divalent metal is calcium. Another suitable divalent metal is magnesium. Also contemplated is zinc. The divalent metals are usually used in a boronic acid:metal ratio of substantially 2:1, in order to achieve the preferred monovalent boronate moiety. Salts containing mixtures of divalent metals, e.g. mixtures of alkaline earth metals, are contemplated by the invention but less preferred.

The invention includes products (compositions of matter) which comprise salts which may be represented by the formula (VI):



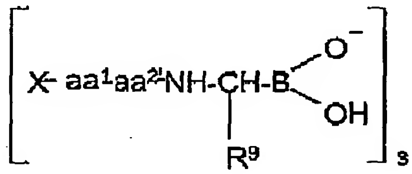
(VI)

where M^{2+} is a divalent metal cation, e.g. an alkaline earth metal or zinc cation, and aa^1, $aa^{2'}$, X and R^9 are as defined above, as well as salts in which both hydroxy groups of the boronate group are deprotonated (preferably with another identical M^{2+} ion) and mixtures of such salts. As previously indicated, the boronate may comprise a tetrahedral species.

<u>2. Group III metals</u>

Suitable Group III metals include aluminium and gallium. Salts containing mixtures of Group III metals are contemplated by the invention but less preferred.

The invention includes products comprising salts of the formula (VII):



(VII)

where M^{3+} is a Group III metal ion and aa^1, $aa^{2'}$, X and R^9 are as defined above, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical M^{3+} group) and mixtures of such salts. As previously indicated, the boronate may comprise a tetrahedral species.

All the salts of the invention in solid form may contain a solvent, e.g. water.

Use of the Products of the Invention

The salts of the invention are useful for formulations, especially for oral formulations, for administering the drug part of the salt. Typically, they are useful as protease inhibitors.

<u>The Salts of the Boronic Acids of Formula III</u>

The salts of the boronic acids of formula III are potent thrombin inhibitors. They are therefore useful for inhibiting thrombin. The invention therefore provides compounds which have potential for controlling haemostasis and especially for inhibiting coagulation, for example preventing secondary events after myocardial infarction. The medical use of the compounds may be prophylactic (including to prevent occurrence of thrombosis) as well as therapeutic (including to prevent re-occurrence of thrombosis or secondary thrombotic events).

Those salts may be employed when an anti-thrombogenic agent is needed. They are thus indicated in the treatment or prophylaxis of thrombosis and hypercoagulability in blood and tissues of animals including man.

It is known that hypercoagulability may lead to thrombo-embolic diseases. Conditions associated with hypercoagulability and thrombo-embolic diseases which may be mentioned include activated protein C resistance, such as the factor V-mutation (factor V Leiden), and inherited or acquired deficiencies in antithrombin III, protein C, protein S, heparin cofactor II. Other conditions known to be associated with hypercoagulability and thrombo-embolic disease include circulating antiphospholipid antibodies (Lupus anticoagulant), homocysteinemi, heparin induced

thrombocytopenia and defects in fibrinolysis. The thrombin inhibitors of the invention are thus indicated both in the therapeutic and/or prophylactic treatment of these conditions.

Particular disease states which may be mentioned include the therapeutic and/or prophylactic treatment of venous thrombosis and pulmonary embolism.

The thrombin inhibitors of the invention are further indicated in the treatment of conditions where there is an undesirable excess of thrombin without signs of hypercoagulability, for example in neurodegenerative diseases such as Alzheimer's disease. In addition to its effects on the coagulation process, thrombin is known to activate a large number of cells (such as neutrophils, fibroblasts, endothelial cells and smooth muscle cells). Therefore, the compounds of the invention may also be useful for the therapeutic and/or prophylactic treatment of idiopathic and adult respiratory distress syndrome, pulmonary fibrosis following treatment with radiation or chemotherapy, septic shock, septicaemia, inflammatory responses, which include, but are not limited to, edema, acute or chronic atherosclerosis such as coronary arterial disease, cerebral arterial disease, peripheral arterial disease, reperfusion damage, and restenosis after percutaneous trans-luminal angioplasty (PTA).

Moreover, the thrombin inhibitors of the invention are expected to have utility in prophylaxis of re-occlusion (i.e. thrombosis) after thrombolysis, percutaneous trans-luminal angioplasty (PTA) and coronary bypass operations; the prevention of re-thrombosis after microsurgery and vascular surgery in general. Further indications include the therapeutic and/or prophylactic treatment of disseminated intravascular coagulation caused by bacteria, multiple trauma, intoxication or any other mechanism; anticoagulant treatment when blood is in contact with foreign surfaces in the body such as vascular grafts, vascular stents, vascular catheters, mechanical and biological prosthetic valves or any other medical device; and anticoagulant treatment when blood is in contact with medical devices outside the body such as during cardiovascular surgery using a heart-lung machine or in haemodialysis.

The salts of the thrombin inhibitors may also be useful in the treatment of pancreatitis.

The salts of the boronic acids of formula III are further considered to be useful for inhibiting platelet procoagulant activity. The invention provides a method for inhibiting platelet procoagulant activity by administering a salt of a formula III boronic acid to a mammal at risk of, or suffering from, arterial thrombosis, particularly a human patient. Also provided is the use of such salts for the manufacture of medicaments for inhibiting platelet procoagulant activity.

The use of the formula III products as inhibitors of platelet pro-coagulant activity is predicated on the observation that they are effective at inhibiting arterial thrombosis as well as venous thrombosis.

Indications involving arterial thrombosis include acute coronary syndromes (especially myocardial infarction and unstable angina), cerebrovascular thrombosis and peripheral arterial occlusion and arterial thrombosis occurring as a result of atrial fibrillation, valvular heart disease, arterio-venous shunts, indwelling catheters or coronary stents. Accordingly, in another aspect the invention provides a method of treating a disease or condition selected from this group of indications, comprising administering to a mammal, especially a human patient, a salt of the invention. The invention includes products for use in an arterial environment, e.g. a coronary stent or other arterial implant, having a coating which comprises a salt of the invention.

The salts of the formula III boronic acids may be used prophylactically to treat an individual believed to be at risk of suffering from arterial thrombosis or a condition or disease involving arterial thrombosis or therapeutically (including to prevent re-occurrence of thrombosis or secondary thrombotic events).

Administration and Pharmaceutical Formulations

The salts may be administered to a host, for example, in the case where the drug has anti-thombogenic activity, to obtain an anti-thrombogenic effect. The boropeptides of formula III have anti-thrombogenic activity. In the case of larger animals, such as humans, the compounds may be administered alone or in combination with pharmaceutically acceptable diluents, excipients or carriers. The term "pharmaceutically acceptable" includes acceptability for both human and veterinary purposes, of which acceptability for human pharmaceutical use is preferred. In the case of oral administration, the compounds are preferably administered in a form which prevents the salt of the invention from contact with the acidic gastric juice, such as enterically coated formulations, which thus prevent release of the salt of the invention until it reaches the duodenum.

The enteric coating is suitably made of carbohydrate polymers or polyvinyl polymers, for example. Examples of enteric coating materials include, but are not limited to, cellulose acetate phthalate, cellulose acetate succinate, cellulose hydrogen phthalate, cellulose acetate trimellitate, ethyl cellulose, hydroxypropyl-methylcellulose phthalate, hydroxypropylmethylcellulose acetate succinate, carboxymethyl ethylcellulose, starch acetate phthalate, amylose acetate phthalate, polyvinyl acetate phthalate, polyvinyl butyrate phthalate, styrene-maleic acid copolymer, methyl-acrylate-methacrylic acid copolymer (MPM-05), methylacrylate-methacrylic acid-methylmethacrylate copolymer (MPM-06), and methylmethacrylate-methacrylic acid co-polymer

(Eudragit® L & S). Optionally, the enteric coating contains a plasticiser. Examples of the plasticiser include, but are not limited to, triethyl citrate, triacetin, and diethyl phthalate.

The anti-thrombotic salts of the invention may also be combined and/or co-administered with any antithrombotic agent with a different mechanism of action, such as the antiplatelet agents acetylsalicylic acid, ticlopidine, clopidogrel, thromboxane receptor and/or synthetase inhibitors, fibrinogen receptor antagonists, prostacyclin mimetics and phosphodiesterase inhibitors and ADP-receptor (P_2 T) antagonists.

The anti-thrombotic salts of the invention may further be combined and/or co-administered with thrombolytics such as tissue plasminogen activator (natural, recombinant or modified), streptokinase, urokinase, prourokinase, anisoylated plasminogen-streptokinase activator complex (APSAC), animal salivary gland plasminogen activators, and the like, in the treatment of thrombotic diseases, in particular myocardial infarction.

The anti-thrombotic salts of the invention may be combined and/or co-administered with a cardioprotectant.

Typically, therefore, the salts of the formula (III) acids may be administered to a host to obtain a thrombin-inhibitory effect.

Actual dosage levels of active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular patient, compositions, and mode of administration. The selected dosage level will depend upon the activity of the particular compound, the severity of the condition being treated and the condition and prior medical history of the patient being treated. However, it is within the skill of the art to start doses of the compound at levels lower than required for to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

According to a further aspect of the invention there is thus provided an oral pharmaceutical formulation including a product of the invention, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is typically mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or one or more: a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol and silicic acid; b) binders such as carboxymethylcellulose, alginates, gelatin,

polyvinylpyrrolidone, sucrose and acacia; c) humectants such as glycerol; d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates and sodium carbonate; e) solution retarding agents such as paraffin; f) absorption accelerators such as quaternary ammonium compounds; g) wetting agents such as cetyl alcohol and glycerol monostearate; h) absorbents such as kaolin and bentonite clay and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycol, for example.

Suitably, the oral formulations may contain a dissolution aid. The dissolution aid is not limited as to its identity so long as it is pharmaceutically acceptable. Examples include nonionic surface active agents, such as sucrose fatty acid esters, glycerol fatty acid esters, sorbitan fatty acid esters (e.g., sorbitan trioleate), polyethylene glycol, polyoxyethylene hydrogenated castor oil, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene alkyl ethers, methoxypolyoxyethylene alkyl ethers, polyoxyethylene alkylphenyl ethers, polyethylene glycol fatty acid esters, polyoxyethylene alkylamines, polyoxyethylene alkyl thioethers, polyoxyethylene polyoxypropylene copolymers, polyoxyethylene glycerol fatty acid esters, pentaerythritol fatty acid esters, propylene glycol monofatty acid esters, polyoxyethylene propylene glycol monofatty acid esters, polyoxyethylene sorbitol fatty acid esters, fatty acid alkylolamides, and alkylamine oxides; bile acid and salts thereof (e.g., chenodeoxycholic acid, cholic acid, deoxycholic acid, dehydrocholic acid and salts thereof, and glycine or taurine conjugate thereof); ionic surface active agents, such as sodium laurylsulfate, fatty acid soaps, alkylsulfonates, alkylphosphates, ether phosphates, fatty acid salts of basic amino acids; triethanolamine soap, and alkyl quaternary ammonium salts; and amphoteric surface active agents, such as betaines and aminocarboxylic acid salts.

The active compounds may also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan and mixtures thereof. Besides inert diluents, the oral compositions may also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavouring and perfuming

agents. Suspensions, in addition to the active compounds, may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminium metahydroxide, bentonite, agar-agar, and tragacanth and mixtures thereof.

The product of the invention may be presented as solids in finely divided solid form, for example they may be micronised.

The active compound may be given as a single dose, in multiple doses or as a sustained release formulation.

Synthesis

1. Peptide/Peptidomimetic Synthesis

The synthesis of boropeptides, including, for example, Cbz-D-Phe-Pro-BoroMpg-OPinacol is familiar to those skilled in the art and described in the prior art mentioned above, including Claeson et al (US 5574014 and others) and Kakkar et al (WO 92/07869 and family members including US 5648338). It is described also by Elgendy et al *Adv. Exp. Med. Biol. (USA)* **1993**, *340*, 173-178; Claeson,G. et al *Biochem.J.* **1993**, *290*, 309-312; Deadman et al *J. Enzyme Inhibition* **1995**, *9*, 29-41, and by Deadman et al *J. Med. Chem.* **1995**, *38*, 1511-1522.

Stereoselective synthesis with S or R configuration at the chiral B-terminal carbon may be conducted using established methodology (Elgendy et al *Tetrahedron. Lett.* **1992**, *33*, 4209-4212; WO 92/07869 and family members including US 5648338) using (+) or (—)- pinanediol as the chiral director (Matteson et al *J. Am. Chem. Soc.* **1986**, *108*, 810-819; Matteson et al *Organometallics..* **1984**, *3*, 1284-1288). Another approach is to resolve the requisite aminoboronate intermediate (e.g. Mpg-BOPinacol) to selectively obtain the desired (R)-isomer and couple it to the dipeptide moiety (e.g. Cbz-(R)-Phe-(S)-Pro, which is the same as Cbz-D-Phe-L-Pro) which will form the remainder of the molecule.

The reader is referred also to other prior documents mentioned previously in this specification, for example the US patents of Adams et al.

The boropeptides may be synthesised initially in the form of boronic acid esters, particularly esters with diols. Such diol esters may be converted to the peptide boronic acid as described next.

2. Ester to Acid Conversion

A boronate ester such as Cbz-D-Phe-Pro-BoroMpg-OPinacol may be hydrolysed to form the corresponding acid, for example as described in Example 1 below, Section H.

A novel technique for converting a diol ester of a peptide boronic acid, especially of formula (III), into the acid comprises dissolving the diol ester in an ether and particularly a dialkyl ether, reacting the thus-dissolved diol with a diolamine, for example a dialkanolamine, to form a product precipitate, recovering the precipitate, dissolving it in a polar organic solvent and reacting the thus-dissolved product with an aqueous acid to form the peptide boronic acid. The boronic acid may be recovered from the organic layer of the mixture resulting from the reaction, for example by removing the solvent, e.g. by evaporation under vacuum or distillation. The reaction between the diol ester and the diolamine may be carried out under reflux, for example.

The identity of the diol is not critical to the invention. As suitable diols may be mentioned aliphatic and aromatic compounds having hydroxy groups that are substituted on adjacent carbon atoms or on carbon atoms substituted by another carbon. That is to say, suitable diols include compounds having at least two hydroxy groups separated by at least two connecting carbon atoms in a chain or ring. A particularly preferred diol is pinacol and other exemplary diols include pinanediol (also a preferred diol), neopentylglycol, diethanolamine, 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, 5,6-decanediol and 1,2-dicyclohexylethanediol.

The alkyl groups of the dialkyl ether preferably have 1, 2, 3 or 4 carbon atoms and the alkyl groups may be the same or different. A most preferred ether is diethyl ether.

The alkyl groups of the dialkanolamine preferably have 1, 2, 3 or 4 carbon atoms and the alkyl groups may be the same or different. A most preferred dialkanolamine is diethanolamine.

The polar organic solvent is preferably $CHCl_3$.

The aqueous acid is suitably a strong inorganic acid at a pH in the region of 1; hydrochloric acid is most preferred.

After reaction with the acid, the reaction mixture is suitably washed with, for example, NH_4Cl .

A preferred procedure is as follows

1. The pinacol ester of the selected peptide boronic acid is dissolved in diethylether.
2. Diethanolamine is added and the mixture is refluxed at 40 °C.
3. The precipitated product is removed, washed (usually several times) with diethylether and dried (e.g. by evaporation under vacuum).

4. The dry product is dissolved in $CHCl_3$. Hydrochloric acid (pH 1) is added and the mixture is stirred approximately 1h at room temperature.

5. The organic layer is removed and washed with NH_4Cl solution.

6. The organic solvent is distilled off and the residual solid product is dried.

The above process when applied to boronic acids of formula III results in the formation of an ester-amide of the peptide boronic acids of formula (I), especially ester-amides with diethanolamine, and such ester-amides are themselves included in the invention.

The invention provides also the use of an organoboronic acid, especially a peptide boronic acid of formula (III) to make a salt of the invention. Included also is a method of preparing a product of the invention, comprising contacting an organoboronic acid, especially a peptide boronic acid of formula (III) with a base capable of making such a salt.

The acid, e.g. peptide boronic acid of formula (III) used to prepare the pharmaceutical preparations is typically of GLP or GMP quality, or in compliance with GLP (good laboratory practice) or GMP (good manufacturing practice); such acids are included in the invention.

Similarly the acids are usually sterile and/or acceptable for pharmaceutical use, and one aspect of the invention reside in a composition of matter which is sterile or acceptable for pharmaceutical use, or both, and comprises a peptide boronic acid of formula (III). Such a composition of matter may be in particulate form or in the form of a liquid solution or dispersion.

The intermediate acid may be in isolated form and such isolated acids are included in the invention, especially isolated acids which are a peptide boronic acid of formula (VIII):

$$X\text{-}(R)\text{-}Phe\text{-}(S)\text{-}Pro\text{-}(R)\text{-}Mpg\text{-}B(OH)_2 \qquad (VIII)$$

wherein X is H (to form NH_2) or an amino-protecting group.

One typical way of providing the intermediate acids is as a particulate composition consisting predominantly of such a peptide boronic acid, and these compositions are included in the invention. The peptide boronic acid often forms at least 75% by weight of the composition and typically at least 85% by weight of the composition, e.g. at least 95% by weight of the composition.

Another typical way of providing the intermediate acids is as a liquid composition consisting of, or consisting essentially of, a peptide boronic acid of formula (II) and a liquid vehicle in which it is

dissolved or suspended. The liquid vehicle may be an aqueous medium, e.g. water, or an alcohol, for example methanol, ethanol, isopropanol, or another propanol, another alkanol or a mixture of the aforegoing.

The compositions of the intermediate acids are generally sterile. The compositions may contain the peptide boronic acid in finely divided form, to facilitate further processing.

3. Salt Synthesis

The salts may be prepared by contacting the relevant boronic acid with the metal hydroxide (alternatively, metal carbonates might be used, for example). Sometimes it is more convenient to contact the acid with the relevant metal alkoxide (e.g. methoxide), for which purpose the corresponding alkanol is a suitable solvent. The preferred salts of the invention are acid salts (one -BOH proton replaced) and, to make these salts, the acid and the base are usually reacted in substantially in the appropriate stoichiometric quantities.

In one procedure, a solution of the peptide boronic acid in a water-miscible organic solvent, for example acetonitrile or an alcohol (e.g. ethanol, methanol, a propanol, especially iso-propanol, or another alkanol), is combined with an aqueous solution of the base. The acid and the base are allowed to react and the salt is recovered. The reaction is typically carried out at ambient temperature (e.g. at a temperature of from 15 to 25°C), but an elevated temperature may be used, for example up to the boiling point of the reaction mixture but more usually lower, e.g. a temperature of up to 40°C or 50°C. The reaction mixture may be allowed to stand or be agitated (usually stirred).

The time during which the acid and the base are allowed to react is not critical but it has been found desirable to maintain the reaction mixture for at least one hour. A period of from one to two hours is usually suitable but longer reaction times are included in the invention.

The salt may be recovered from the reaction mixture by any suitable method, for example evaporation, precipitation or crystallisation. In one preferred technique, the salt is recovered by evacuating the reaction mixture to dryness. The salt is preferably thereafter purified, for example by redissolving the salt before filtering the resulting solution and drying it, for example by evacuating it to dryness or freeze drying. The redissolution may be performed using water, e.g. distilled water. The salt may then be further purified, for example in order to remove residual water by further redissolution in a suitable solvent, which is advantageously ethyl acetate or THF followed by evaporating to dryness. The purification procedure may be carried out at ambient temperature (say, 15 to 25°C), or at a modestly elevated temperature, such as

e.g. a temperature not exceeding 40°C or 50°C; for example the salt may be dissolved in water and/or solvent by agitating with or without warming to, for example, 37°C.

The invention includes a method for drying the salts of the invention and other peptide boronic acid salts, comprising dissolving them in ethyl acetate or THF and then evaporating to dryness, e.g. by evacuation.

Generally, preferred solvents for use in purifying the salts are ethyl acetate or THF, or perhaps another non-polar solvent.

A general procedure for synthesising multivalent metal salts of Cbz-Phe-Pro-BoroMpg-OH is as follows:

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added the requisite base as a solution in distilled water (190ml) [0.1M solution for a divalent metal; 0.67M solution for a trivalent metal]. The resultant clear solution is allowed to react for example by being left to stand or being agitated, for a usual period, in either case, of from one to two hours. The reaction is typically carried out at ambient temperature (e.g. 15-25°C) but alternatively the temperature may be elevated (e.g. up to 30°C, 40°C or 50°C). The reaction mixture is then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in the minimum amount of distilled water necessary (200ml to 4L), typically with warming (e.g. to 30-40°C), usually for up to 2 hours. The solution is filtered, suitably through filter paper, and evacuated to dryness, again with the temperature of the solution not exceeding 37°C, or freeze dried. The resultant product is dried under vacuum overnight to normally yield a white brittle solid. If the product is present as an oil or tacky solid then it is dissolved in ethyl acetate and evacuated to dryness to produce the product as a white solid. The white solid is typically a coarse, amorphous powder.

In variations of the aforegoing general procedure, the acetonitrile is replaced by another water-miscible organic solvent, notably an alcohol, as discussed above, especially ethanol, methanol, iso-propanol or another propanol.

The above synthetic procedures are applicable also to preparing an alkali metal salt of TRI50c, which is useful as a starting material for alternative syntheses of multivalent metal salts, where direct synthesis from the acid is not ideal, as in the case of excessively insoluble multivalent metal hydroxides. In such an "indirect" synthesis from an alkali metal salt, especially the sodium salt or alternatively the potassium salt, the boronic acid salt in solution is contacted with a salt of the relevant metal (normally a salt having a pharmaceutically acceptable anion, e.g. chloride).

The multivalent metal salt of the boronic acid is then recovered, for example it will often precipitate out (when the multivalent metal salt is less soluble in the reaction medium than is the sodium salt). The resulting precipitate may then be separated from the liquid, e.g. by filtration, and purified.

The preparation of the multivalent metal salts of the invention from the corresponding alkali metal salts is novel and included in the invention. The alkali metal salts and their aqueous solutions also form part of the invention.

Separation of Stereoisomers

The stereoisomers of a peptide boronic acid or a synthetic intermediate aminoboronate may be resolved in, for example, any known way. Accordingly, they may be resolved by chromatography (HPLC) or salt crystallisation.

Examples

The following compounds are referred to in the Examples:

TRI50b = Cbz-Phe-Pro-BoroMpg-Opinacol.
TRI50c = Cbz-Phe-Pro-BoroMpg-OH. This is the free acid of TRI50b.

It is considered that the TRI50b and TRI50c featured in the examples are at least predominantly of the most active isomer, considered to be of RSR (DLL) configuration, as discussed above.

The solubility data presented in the examples were obtained from salt made using a modification of the salt preparation process described in the examples. The modified process differs from that described in the examples in that 100mg of TRI50c was used as starting material, the product of the redissolution in water was dried by freeze drying and the filtration was carried out through a 0.2µm filter. The salt for which solubility data are presented is believed to contain about 85% of the most active isomer, considered to be of RSR configuration. When repeated with very pure active isomer salt obtained using the procedure described in the example from isomerically pure TRI50c, the solubility data were the same as those presented within experimental error or very slightly higher.

<u>EXAMPLE 1 – SYNTHESIS OF TRI50C</u>

<u>A. 3-METHOXYPROPENE</u>

1. REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

1,4-Dioxan (SPS).

Toluene, AR grade.

Allyl Alcohol.

Sodium Hydride as 60% dispersion in mineral oil. It should be a pale grey powder. Overall white colour indicates decomposition.

Dimethyl sulphate.

Magnesium sulphate dried (SLR).

Water, standard laboratory purified water is used throughout.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

1.2 PURIFICATION OF REAGENTS

All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of dry nitrogen before use.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure.

All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

The mechanical stirrer should be of sufficient torque to stir a viscous suspension. The stirrer arm should be fitted to the flask through a quickfit sleeve with inert oil seal.

Reaction is conducted in a three necked flask, to allow overhead stirring, inert gas purge and sodium hydride addition. A heating mantle of appropriate size is required.

3 PROCEDURE

3.1 PREPARATION

To a mechanically stirred cooled solution under nitrogen with a gas outlet and fitted with a water condenser of allyl alcohol (107.8ml, 1.59mol) and dimethylsulphate (200ml, 1.59mol, 1.eq.) in 1,4-dioxane (1L) is added, portionwise NaH (60% dispersion in mineral oil, 63.5g, 1.59mol, 1eq.). Care is taken that the reaction temperature remains at or below room temperature and the reaction is stirred until effervescence has ceased.

3.2 PURIFICATION AND WORK-UP

The slurry is stirred, carefully, into ice (1L), and extracted with toluene (3x500ml). The organic phase is heated (mantle) with a fractionation column, to distil off at atmospheric pressure the methoxypropene, b.p. 45-60^{o}C. Heating should be observed to keep the vapour temperature in the 45-60^{o}C range, since unreacted allyl alcohol distils at 96-98^{o}C.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The distilled 3-methoxypropene should be checked by ^{1}H NMR spectroscopy.

B. 3-METHOXYPROPYL BORONATE CATECHOL ESTER

1 REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

Catecholborane. The appearance should be a low melting (m.p. 12^{o}C) solid.

3-methoxypropene. The appearance should be a clear volatile liquid. It must be stored at below 4^{o}C.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure.

A heat gun or water bath is required to prewarm the bottle of catecholborane.

All glassware must be heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

To 3-methoxypropene (120g, 1.66mol) in a 1l flask cooled in an ice bath and fitted with a condenser, is added, dropwise by dry transfer via a dropping funnel, catecholborane (199.6g, 1eq.) (which is prewarmed, if necessary, to give a liquid) and left overnight at room temperature. Careful addition of the catecholborane is necessary as the reaction can become violently exothermic.

The mixture is heated at 60-70°C for 24hrs. The mixture is allowed to cool to room temperature.

3.2 PURIFICATION AND WORK-UP

There is no purification at this stage. Used immediately.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The catechol 3-methoxypropyl boronate should be checked by ^{1}H NMR spectroscopy. °C Signals should be observed as follows:-

δ_{60}	Signal Pattern	Assignment
7.13	4H, multiplet	Ph
3.4	2H, multiplet	$\underline{CH_2}$Ome
3.39	3H, singlet	O$\underline{me}$
1.92	2H, multiplet	$\underline{CH_2}$
1.29	2H, multiplet	$\underline{CH_2}$

Observation of other signals would be indicative of impurities

C. 3-METHOXYPROPYL BORONATE PINACOL ESTER

1 REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

Pinacol.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure.

All glassware must be heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

To catechol 3-methoxypropaneboronate (1.66mol, from section B2) is added, at 0°C, pinacol (126g, 1eq). The solution is stirred at 0°C for 1hr. Remove the ice bath and leave at room temperature overnight.

3.2 PURIFICATION AND WORK-UP

To a 3l flask containing 1.5l hexane (lab. grade, not dried) transfer the solution from 3.1. Allow the catechol to precipitate out (storage at <4°C for 1-2 hrs. facilitates this) and decant off the hexane into a 3l separating funnel. Wash the precipitate with a further 500ml of hexane and add to the first hexane solution. Wash the hexane with water (2x500ml, analytical grade). Back extract each aqueous wash with (2x500ml) hexane. Dry the hexane layer with anhydrous $MgSO_4$. Filter (glass sinter, grade four).

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The pinacol 3-methoxypropyl boronate product should be checked by ^1H NMR spectroscopy oC. Signals should be observed as follows:-

δ_{400}	δ_{60}	Signal Pattern	Assignment
3.33-3.37	-	5H, multiplet	$C\underline{H}_2$-O-$C\underline{H}_3$
1.69	-	2H, multiplet	CH_2 $C\underline{H}_2$
1.24	-	12H, singlet	pinacol
0.79	-	2H, multiplet	$C\underline{H}_2B$

Due to the presence of impurities other signals will be observed also.

If impurity levels are unacceptable, distil the product (bp. 55°C/0.4mmHg, pinacol 3-methoxypropyl boronate).

D. 4-METHOXY-1-CHLOROBUTYL BORONATE PINACOL ESTER

1 REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

Dichloromethane (AR) dried/redistilled before use.

Tetrahydrofuran (AR) dried/redistilled before use.

Hexane (AR).

Lithium diisopropylamide, 2.0M in hexane/tetrahydrofuran/ethylbenzene. The reagent must be inspected before each use. It should be a clear pale red/brown solution. If it deviates from this colour or has any white precipitate it must be discarded. Store at <6°C.

Zinc chloride, 0.5M in THF.

Cyclohexane, anhydrous, 99.5%.

Benzophenone (SLR).

Sodium metal stored under paraffin oil (SLR).

Phosphorus pentoxide (SLR).

Magnesium sulphate dried (SLR).

Water, Ultra Pure grade.

Carbon tetrachloride (GLR)

Dry ice.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

1.2 PURIFICATION OF REAGENTS

All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of argon before use.

1.2.1 Dichloromethane

Add phosphorus pentoxide to the dichloromethane at the rate of ca. 10 g per $100cm^3$ and leave to stand in a stoppered flask for at least 30 minutes. Distil the dichloromethane from the phosphorus pentoxide under a stream of dry nitrogen. The purified solvent is used immediately

1.2.2 Tetrahydrofuran

The distillation apparatus is normally set up in the laboratory ready for use and will contain tetrahydrofuran over sodium containing benzophenone (ca. 0.5 g per litre) as an indicator. If necessary top up the distillation flask with more tetrahydrofuran so that it is at most two thirds full. If the colour of the solvent in the distillation flask is not blue add sodium (in oil) in small pieces, ca. 5 mm cubes until a blue colour develops. Distil the solvent from the sodium under a stream of dry nitrogen.

The purified tetrahydrofuran is used immediately and stored.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents are used for this preparation procedure.

All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

To a solution (0.4M, in a 10l flask) of pinacol 3-methoxypropylboronate ester (150g, 0.750mol) in anhydrous cyclohexane (1250ml) and THF (625ml) (section 1.2.2) cooled to -20°C in a carbon tetrachloride/dry ice bath, is added dry DCM (section 1.2.1, 1.22eq., 58.5ml, 0.915mol). Added to this solution (with stirring, under stream of dry argon) dropwise, to maintain the temperature between -20 °C and -15 °C, is lithium diisopropylamide (1.11eq., 416ml, 0.833mol, diluted in 500ml THF) and then zinc chloride (0.5M solution in THF,1500ml) pre cooled in ice. The reaction is allowed to warm to room temperature overnight.

3.2 PURIFICATION AND WORK-UP

The reaction mixture is diluted in hexane (2l) and poured into cold 1M sulphuric acid (1l), stir for 15 mins, and then extract with hexane (2x500ml). Wash the combined extracts with saturated $NaHCO_3$ solution (1l), saturated NaCl solution (1l). Dry the combined hexane extracts with anhydrous $MgSO_4$.

Filter immediately with a grade four glass sinter.

Remove the solvent using a rotary evaporator at room temperature and with a vacuum of ca. 1 mm/Hg. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The unpurified pinacol 4-methoxy-1-chlorobutylboronate should be checked by ^1H NMR spectroscopy. Signals should be observed as follows:-

δ_{400}	Signal Pattern	Assignment
3.47-3.38	3H, multiplet	CH_2Ome and CHB
3.34	3H, singlet	Ome
2.0-1.62	4H, multiplet	CH_2CH_2
1.27	12H, singlet	pinacol

Due to the presence of impurities other signals will be observed also.

E. 4-METHOXY-1-BIS (TRIMETHYLISILYL) AMINOBUTYL BORONATE PINACOL ESTER

1 REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

Tetrahydrofuran (AR) dried/redistilled before use.

n-Hexane SPS grade dried/redistilled before use.

Lithium bis(trimethylsilyl)amide, 1N solution in anhydrous hexane.

Water, Ultra Pure grade.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

1.2 PURIFICATION OF REAGENTS

All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of dry nitrogen before use.

1.2.1 Tetrahydrofuran

The distillation apparatus is normally set up in the laboratory ready for use and will contain tetrahydrofuran over sodium containing benzophenone (ca. 0.5 g per litre) as an indicator. If necessary top up the distillation flask with more tetrahydrofuran so that it is at least two thirds

full. If the colour of the solvent in the distillation flask is not blue add sodium in oil in small pieces, ca. 5 mm cubes, until a blue colour develops. Distil the solvent from the sodium under a stream of dry nitrogen.

The purified tetrahydrofuran is used immediately and not stored.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure. All glassware must be heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

A 0.33M solution of pinacol 4-methoxy-1-chlorobutaneboronate (150g, 0.60mol) in THF (1810ml) is added to a 0.5M solution of lithium hexamethyldisilazane (1N in hexane, 604ml, 1eq) in THF (603ml) at -78°C (dry ice/acetone bath) giving a final concentration of boronate at 0.2M. The reaction mixture is allowed to warm slowly to room temperature and is stirred for at least 12hrs.

3.2 PURIFICATION AND WORK-UP

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

Hexane (lab grade, 1000ml) is added to yield a precipitate which is removed by washing with water (2x750ml, analytical grade). Back extract each aqueous phase with (500ml) hexane. Dry the hexane layer with anhydrous $MgSO_4$ and filter through a grade 4 glass sinter. The organic phase is concentrated using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

The residual oil is distilled under reduced pressure to give b.p. 80-104°C, 0.1 – 0.2 mmHg pinacol 4-methoxy-1-bis(trimethylsilyl)aminobutyl boronate.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The distilled pinacol 4-methoxy-1-bis(trimethylsilyl)aminobutyl boronate should be checked by ^1H NMR spectroscopy °C. Signals should be observed as follows:-

δ_{400}	Signal Pattern	Assignment
3.23-3.26	5H, multiplet	$C\underline{H}_2O$ $C\underline{H}_3$
2.41	1H, multiplet	CH_2 $C\underline{H}B$
1.62	2H, multiplet	$C\underline{H}_2$ CH_2
1.46	1H, multiplet	1H from $C\underline{H}_2$ (split H)
1.31	1H, multiplet	1H from $C\underline{H}_2$ (split H)
1.12	12H, singlet	pinacol

Due to the presence of impurities other signals will be observed also.

F. 4-METHOXY-1-AMINOBUTYL BORONATE PINACOL ESTER

1. REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

n-Hexane SPS grade dried/redistilled before use.

Chloroform (AR) dried/redistilled before use.

HCL, 4N anhydrous solution in 1,4-dioxan.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

1.2 PURIFICATION OF REAGENTS

All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of dry nitrogen before use.

1.2.1 n-Hexane

Add calcium hydride to the n-hexane at the rate of ca. 10 g per $100cm^3$ and leave to stand in a stoppered flask for at least 30 minutes. Distil the hexane from the calcium hydride under a

stream of dry nitrogen. The purified solvent should be used immediately wherever possible but may be stored for up to 24 hours in a tightly stoppered flask.

1.2.2 Chloroform.

Add phosphorus pentoxide to the chloroform at the rate of ca. 10 g per $100cm^3$ and leave to stand in a stoppered flask for at least 30 minutes. Distil the chloroform from the phosphorus pentoxide under a stream of dry nitrogen. The purified solvent should be used immediately wherever possible but may be stored for up to 24 hours in a tightly stoppered flask.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure

All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

To a 0.4M solution of pinacol 4-methoxy-1-bis(trimethylsilyl)aminobutane boronate (160g, 0.428mol) in dry hexane (1072ml, section 1.2.1) at -78°C (dry ice/acetone), is added HCl(4N, solution in dioxane, 322ml, 3eq.) from a measuring cylinder. The reaction is allowed to warm to room temperature overnight.

3.2 PURIFICATION AND WORK-UP

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

Dry chloroform (2l, section 1.2.2) is added. The solution is then filtered through celite under nitrogen pressure in a closed system(grade four glass sinter). Organic phase is concentrated using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

Pinacol 4-methoxy-1-aminobutyl boronate should be checked by electrospray mass spectrometry. The signals observed should be:

Signal (AMU)	Assignment
253	[M+Na]
230	[M+]

Due to the presence of impurities other signals will be observed also.

<u>G. Cbz-D-Phe-Pro-BoroMpg-Opinac (TRI50b)</u>

1.REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

Tetrahydrofuran (AR) dried/redistilled before use.

n-Hexane SPS grade.

Isobutylchloroformate.

N-methylmorpholine.

Triethylamine.

Benzophenone (SLR).

Sodium Chloride (SLR).

Sodium bicarbonate (SLR).

Hydrochloric Acid (SLR).

Sodium metal stored under paraffin oil (SLR).

Magnesium sulphate dried (SLR).

Water, Ultra Pure grade.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self-indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

1.2 PURIFICATION OF REAGENTS

All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of dry nitrogen before use.

1.2.1 Tetrahydrofuran

The distillation apparatus is normally set up in the laboratory ready for use and will contain tetrahydrofuran over sodium containing benzophenone (ca. 0.5 g per litre) as an indicator. If necessary top up the distillation flask with more tetrahydrofuran so that it is at least two thirds full. If the colour of the solvent in the distillation flask is not blue add sodium in oil in small pieces, ca. 5 mm cubes, until a blue colour develops. Distil the solvent from the sodium under a stream of dry nitrogen.

The purified tetrahydrofuran is used immediately and not stored.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure.

All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

To a 0.5M solution of Cbz-D-Phe-Pro (0.515mol,204.5g,1eq) in THF (1042ml) is added N-methylmorpholine (56.8ml, 1eq.) and the solution cooled to -20°C (CCl_4/dry ice bath). $^iBuOCOCl$ (67ml,1eq, in 149ml THF, 3.5M) is added making sure the temperature stays in the range of -20 °C to -15°C. After 15 mins, to the mixture, is added by dry transfer a 1.36M solution of pinacol 4-methoxy-1-aminobutylboronate hydrochloride (150g, 0.57mol, 1.05eq) as a precooled solution in $CHCl_3$ (416ml), then Et_3N (75.3ml,1.05eq) is added. The reaction is allowed to warm to room temperature and stirred for at least 2hrs.

3.2 PURIFICATION AND WORK-UP

Remove th solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

The residue is dissolved in ethyl acetate (1500ml) and washed with HCl (0.2M, 2x500ml),back extract the combined HCl washes with ethyl acetate (500ml) and combine with ethyl acetate layer. Wash combined ethyl acetate with water (1000ml), back extract the water wash with 500ml of ethyl acetate combined with ethyl acetate layer, $NaHCO_3$ (saturated aqueous, 2x1000ml) and NaCl (saturated aqueous, 500ml). To the organic phase is added dried magnesium sulphate until it flocculates, the flask stoppered tightly and left to stand for at least 30 minutes. Remove the magnesium sulphate by filtration through a glass sinter, (grade four). Remove the solvent using a rotary evaporator at room temperature and with a vacuum of ca. 1 mm/Hg. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

Leave overnight on high vacuum.

The desired crude product as a foamy solid.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

3.3.1 NMR Analysis

The TRI50b should be checked by ^{1}H NMR spectroscopy. Signals should be observed as follows:-

δ_{400}	Signal Pattern	Assignment
7.82	1H, broad	NH
7.40-7.20	10H, multiplet	2xPh
5.7	1H, broad	NH
5.17-5.08	2H, dd, J=7.54Hz	Ph$\underline{CH_2}$O
4.48-4.44	2H, multiplet	Pro α-CH, Pheα-CH
3.46	1H, multiplet	Pro-C4
3.27	2H, multiplet	$\underline{CH_2}$Ome
3.22	3H, singlet	Ome
2.99	2H, multiplet	Ph$\underline{CH_2}$
2.63	1H, multiplet	CHB

2.59-2.23	4H, multiplet	Pro-C3, Pro-C2
1.60	4H, multiplet	CH_2CH_2
1.20	12H, singlet	pinacol

The TRI50b should be checked by ^{13}C NMR spectroscopy. °C Signals should be observed as follows:-

δ_{400}	Signal Pattern	Assignment
171	quaternary	O-CO-N
156	quaternary	CH-CO-N
136	quaternary	Ph
130-126	CH	aromatics
81.5	quaternary	Cme_2
73	CH_2	CH_2Ome
67.26	CH_2	$PhCH_2O$
58.3	CH	Pro-αCH
57.94	CH_3	Ome
54.46	CH	Phe-αCH
46.77	CH_2	Pro-4-CH_2
38.76	CH_2	$PhCH_2CH$
27.84-27.4	2x CH_2	$CH_2CH_2CH_2Ome$
25.23-24.9	$4xCH_3$	pinacol, major isomer
24.07	CH_2	Pro-3- CH_2

Due to the presence of impurities other signals will be observed also.

3.3.2 HPLC Analysis

[note: a) tripeptide cannot be recovered from aqueous solution. b) Dipeptide elutes at solvent front and does not give a peak in this system]

- Column: Reverse phase C-18 ODS (octadecylsilane) 2.5μm, 150x4.6mm
- Flow: 1.5ml/min.
- Detection: UV at 225 nm
- Injection volume: 0.02ml
- Solvent A: 20% MeCN in analytical grade water.
- Solvent B: 55% MeCN in analytical grade water.

- Gradient: Linear from 20 to 90% mobile phase B over initial 15 minutes. Conditions maintained at 90% mobile phase B for a further 10 minutes. Linear to 100% B over 10mins, conditions maintained at 100% B for 5 mins then re-equilibrated to initial conditions.

Component	Rt (min)
Z-D-Phe-Pro-(S)-boroMpgOPinacol	16(+-1)
Z-D-Phe-Pro-®-boroMpgOPinacol	17(+-1)

H. Cbz-D-Phe-Pro-BoroMpg-OH (TRI50c)

To a solution of TRI50b (rmm 608) in acetone (1g/10ml), is added phenyl boronic acid (1.01 equivalent, rmm 120) and the solution stirred by a mechanical stirrer. To the solution is slowly added ammonium hydroxide solution, (5%, pH adjusted to pH 9 by HCl, same volume as acetone). Some cloudiness may develop.

Hexane (equal volume to total acetone and ammonium hydroxide) is added and the solution stirred rapidly for four hours. Stirring is stopped and the hexane layer decanted (if an oil forms, this is kept with the aqueous layer by washing with a small volume of acetone). Hexane (same volume) is added, stirred for 10mins, decanted and repeated.

The aqueous layer is concentrated to about 1/3 volume by rotary evaporator with card-ice cold finger (water bath <35°C). Some oil may form on the side of the flask. The solution is then acidified (0.1N HCl) to pH 3 (care: do not acidify below pH 3), and extracted by EtOAc (2x same as original acetone volume). Sample can be concentrated without drying to give a foam, yield ~70%.

EXAMPLE 2 – ALTERNATIVE CONVERSION OF TRI50B TO TRI50C

1. Approximately 300 g of TRI50b were dissolved in approximately 2.5 L diethylether.
2. Approximately 54 ml diethanolamine were been added, the mixture was refluxed at 40 °C.
3. The precipitated product was removed, washed several times with diethylether and dried.
4. The dry product was dissolved in $CHCl_3$. Hydrochloric acid (pH 1) was added and the mixture was stirred approximately 1h at room temperature.
5. The organic layer was removed and washed with NH_4Cl solution.
6. The organic solvent was distilled off and the residual solid product was dried.

Typical yield: Approximately 230 g

EXAMPLE 3 - SEPARATION OF DIASTEREOMERS

The R-Mpg and S-Mpg isomers of TRI50b and TRI50c are separated chromatographically as summarised below.

A solution of 5gm/ml of TRI50b in acetonitrile is prepared and 10 μL is injected to a Lichrosphere™ cyano column and eluted with a gradient of n-hexane and tetrahydrofuran with monitoring at 206nM. Analysis of the UV chromatogram indicates TRI50b isomer I ('R' configuration at α-aminoboronate centre) elutes at (retention time) Rt 11.1 minutes; TRI50b isomer II ('S' configuration at α-aminoboronate centre) elutes at Rt 13.7minutes.

Following the same procedure, TRI50c isomer I ('R' configuration at α-aminoboronate centre) elutes at (retention time) Rt 21.2 minutes; TRI50b isomer II ('S' configuration at α-aminoboronate centre) elutes at Rt 22.2 minutes.

Conditions:
Column: Licrosphere Cyano Merck.4.6 x 250mm, 5μ.
Solvent A : n-Hexane
Solvent B THF
Gradient 0-100% B over 25 minutes

Monitor UV at 206nm
Sample concentration 5mg/ml.

The results are shown in the chromatogram of Fig 1.

The above microanalytical data show C and N amounts below calculated, suggesting the samples might have contained unremoved water.

EXAMPLE 4 - PREPARATION OF CALCIUM SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added $Ca(OH)_2$ as a 0.1M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant product is a white brittle solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

Yield: 17.69g.

Microanalysis: See Example 10.

EXAMPLE 5 – UV/VISIBLE SPECTRA OF CALCIUM SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \varepsilon cl$ where A is the absorbance
C is the concentration
l the path length of the UV cell
and ε is the extinction coefficient.

Extinction coefficient: 955.

EXAMPLE 6 – AQUEOUS SOLUBILITY OF CALCIUM SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material.

Solubility when dissolved at 25mg/ml: 5mM (5 mg/ml).

EXAMPLE 7 – *IN VITRO* ACTIVITY OF CALCIUM SALT OF TRI50C

TRI50c calcium salt was assayed as an inhibitor of human α-thrombin by an amidolytic assay (J. Deadman et al J. Med. Chem. 1995, 38, 15111-1522, which reports a Ki value of 7nM for TRI50b).

TRI50c calcium salt was observed to have a Ki of 10nM.

EXAMPLE 8 – PREPARATION OF ZINC SALT OF TRI50C

The relative solubilities of the respective hydroxides of magnesium and zinc are such that, if these hydroxides had been used to prepare the corresponding TRI50c salts using the procedure of Example 4, they would not have resulted in homogeneous salt formation. New methods were therefore developed to prepare the sodium and zinc salts, as described in examples 7 and 8.

TRI50c sodium salt (2.24g, 4.10mM) was dissolved in distilled water (100ml) at room temperature and zinc chloride in THF (4.27ml, 0.5M) was carefully added with stirring. A white precipitate that immediately formed was filtered off and washed with distilled water. This solid was dissolved in ethyl acetate and washed with distilled water (2 x 50ml). The organic solution was evacuated to dryness and the white solid produced dried over silica in a desiccator for 3 days before microanalysis. Yield 1.20g.

^{1}H NMR 400MHz, $\delta_H(CD_3OD)$ 7.23-7.33 (20H, m, ArH), 5.14 (4H, m, $PhCH_2O$), 4.52 (4H, m, αCH), 3.65 (2H, m), 3.31 (12H, m), 3.23 (6H, s, OCH_3), 2.96 (4H, d, J 7.8Hz), 2.78 (2H, m), 2.58 (2H, m), 1.86 (6H, m), 1.40 (10H, m).

^{13}C NMR 75MHz 393K $\delta_C(CD_3OD)$ 178.50, 159.00, 138.05, 137.66, 130.54, 129.62, 129.50, 129.07, 128.79, 128.22, 73.90, 67.90, 58.64, 58.18, 56.02, 38.81, 30.06, 28.57, 28.36, 25.29. FTIR (KBr disc) ν_{max} (cm^{-1}) 3291.1, 3062.7, 3031.1, 2932.9, 2875.7, 2346.0, 1956.2, 1711.8, 1647.6, 1536.0, 1498.2, 1452.1, 1392.4, 1343.1, 1253.8, 1116.8, 1084.3, 1027.7, 916.0, 887.6, 748.6, 699.4, 595.5, 506.5.

EXAMPLE 9 – PREPARATION OF MAGNESIUM SALT OF TRI50C

TRI50c (1.00g, 1.90mM) was dissolved in methanol (10ml) and stirred at room temperature. To this solution was added magnesium methoxide ($Mg(CH_3O)_2$) in methanol (1.05ml, 7.84 wt%). This solution was stirred for 2 hours at room temperature filtered and evacuated to 5ml. Water (25ml) was then added and the solution evacuated down to dryness to yield a white solid. This was dried over silica for 72hrs before being sent for microanalysis. Yield 760mg.

^{1}H NMR 300MHz, $\delta_H(CD_3C(O)CD_3)$ 7.14 – 7.22 (20H, m), 6.90 (2H, m), 4.89 (4H, m, $PhCH_2O$), 4.38 (2H, m), 3.40 (2H, br s), 2.73 – 3.17 (20H, broad unresolved multiplets), 1.05 – 2.10 (16H, broad unresolved multiplets).

^{13}C NMR 75MHz 393K $\delta_C(CD_3C(O)CD_3)$ 206.56, 138.30, 130.76, 129.64, 129.31, 129.19, 129.09, 128.20, 128.04, 74.23, 73.55, 67.78, 58.76, 56.37, 56.03, 48.38, 47.87, 39.00, 25.42, 25.29. FTIR (KBr disc) ν_{max} (cm^{-1}) 3331.3, 3031.4, 2935.3, 2876.9, 2341.9, 1956.1, 1711.6, 1639.9, 1534.3, 1498.1, 1453.0, 1255.3, 1115.3, 1084.6, 1027.6, 917.3, 748.9, 699.6, 594.9, 504.5, 467.8.

EXAMPLE 10 – ANALYSIS OF CALCIUM, MAGNESIUM AND ZINC SALTS OF TRI50C

A. Calcium Salt

Analytical data

HPLC or LC/MS: HPLC betabasic C18 Column, CH_3CN, Water

Estimated Purity: >95% by UV (λ_{215nm})

Micro analysis:

	Calcd.	Found.
C:	59.27	55.08
H:	6.48	6.43
N:	7.71	7.08
Other: B:	1.99	2.01
Ca:	3.68	3.65

Physical Properties

Form: Amorphous solid

Colour: White

Melting Point: N/A

Solubility: Soluble in aqueous media *ca*~4mg/ml

M_w: 1088.89

B. Magnesium Salt

Analytical data

HPLC or LC/MS: HPLC betabasic C18 Column, CH_3CN, Water

Estimated Purity: >90% by UV (λ_{215nm})

Micro analysis:

	Calcd.	Found.
C:	60.44	57.25
H:	6.57	6.71
N:	7.83	7.45
Other: B:	2.01	2.02
Mg:	2.26	2.12

Physical Properties

Form: Amorphous solid

Colour: White

Melting Point: N/A

Solubility: Soluble in aqueous media *ca*~7mg/ml

M_w: 1073.12

C. Zinc Salt

Analytical data

HPLC or LC/MS: HPLC betabasic C18 Column, CH_3CN, Water

Estimated Purity: >95% by UV (λ_{215nm})

Micro analysis:

	Calcd.	Found.
C:	58.21	56.20
H:	6.33	6.33
N:	7.54	7.18
Other: B:	1.94	1.84
Zn:	5.87	7.26

Physical Properties

Form: Amorphous solid

Colour: White

Melting Point: N/A

Solubility: Soluble in aqueous media *ca*~2mg/ml

M_w: 1114.18

Conclusion

The zinc, calcium and magnesium salts have all been prepared with a stoichiometry of one metal ion to two molecules of TRI50c. The values found for the calcium and magnesium salts are close to those calculated for this 1:2 stoichiometry. For the zinc salt an excess of zinc was found.

EXAMPLE 11 - STABILITY

The calcium salt of TRI50c was stored for one month at 40°C and 75% relative humidity. Analysis at the end of the period showed that it contained not more than 1% of a major impurity designated as impurity 1. This indicates that the salt is stable under normal storage conditions.

EXAMPLE 12 – *IN-VITRO* ASSAY AS THROMBIN INHIBITOR OF MAGNESIUM SALT OF TRI50C

Thrombin Amidolytic Assay

TRI50c magnesium salt (TRI 1405) was tested in a thrombin amidolytic assay.

Reagents:

Assay Buffer:
100mM Na phosphate
200mM NaCl (11.688g/l)
0.5% PEG 6000 (5g/l)
0.02% Na azide
pH 7.5

Chromogenic substrate S2238 dissolved to 4mM (25mg + 10ml) in water. Diluted to 50uM with assay buffer for use in assay at 5μM. (S2238 is H-D-Phe-Pip-Arg-pNA).

Thrombin obtained from HTI, via Cambridge Bioscience, and aliquoted at 1mg/ml with assay buffer. Dilute to 100ng/ml with assay buffer and then a further 1 in 3 for use in the assay.

Assay:

110μl assay buffer
50ul 5μg/ml thrombin
20μl vehicle or compound solution

5 min at 37°C

20µl 50µM S2238

Read at 405nm at 37°C for 10minutes and record Vmax

Results:

The results are presented in Fig. 2.

Discussion:

In this assay the magnesium salt of TRI50c shows the same activity as TRI50b as an external control.

EXAMPLE 13 (COMPARATIVE) - PREPARATION OF POTASSIUM SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added KOH as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 1L distilled water with warming to 37°C for about 2 hours. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid.
Yield: 14.45 mg.

The salt was then dried under vacuum over silica to constant weight (72 h).

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)	Metal % Found (Calc.)
54.84 (57.55)	6.25 (6.26)	7.02 (7.45)	2.01 (1.92)	K 4.29 (6.94)

EXAMPLE 14 (COMPARATIVE) – AQUEOUS SOLUBILITY OF POTASSIUM SALT OF TRI50C

The UV/visible spectra of TRI50c and its solubility were obtained as described above in relation to the calcium salt. Solubility when dissolved at 25mg/ml: 29mM (16 mg/ml).

EXAMPLE 15 (COMPARATIVE) – SOLUBILITY OF TRI50C

The UV/visible spectra of TRI50c and its solubility were obtained as described above in relation to the calcium salt. The solubility of TRI50c when dissolved at 50mg/ml was 8mM (4mg/ml).

EXAMPLE 16 – INTRADUODENAL ABSORPTION IN RAT

A. Preparation of Liquid Formulations of TRI50c and Salt

1. Preparation of buffer solution pH 4.5

Place 1.48 g of sodium acetate (anhydrous) in a 1000 mL volumetric flask, add 16 mL 2N CH_3COOH, then add water and mix. Adjust the pH to 4,5 using 0.2 N NaOH and fill up with water.

2. Preparation of buffer solution pH 6.8 (USP)

Place 50.0 mL monobasic potassium phosphate 0.2 M in a 200 mL volumetric flask add 22.4 mL NaOH 0.2 M fill up with dest. Water. Check the pH and adjust if necessary.

3. Preparation of the formulation

- Place 10 mg of the relevant compound in an Eppendorf cup
- Add 0.5 mL ethanol and shake for 10 minutes
- Sonicate for 10 minutes
- Add 1.5 mL of buffer
- Shake for additional 15 minutes
- Resulting target concentration: 5 mg/mL

B. Intraduodenal Studies

The intraduodenal studies were performed using male Wistar rats, approximately 8 weeks of age and weighing between 250 and 300 g.

Food was withheld overnight prior to dosing and returned approximately 2 hours post-dose. Water was available *ad libitum*.

Animals were anaesthetised using gaseous halothane. A small incision was made in the abdomen and the duodenum located. Each animal received a single administration of control or test article by injection directly into the duodenum, using a constant dose volume of 4mL/kg. Following administration the incision was closed using surgical staples.

Individual dose volumes were based on individual body weights, obtained on the day of dosing. Treatments employed for the study were as follows:

Treatment	Dose level (mg/kg)	Formulation concentration (mg/mL)	Number of animals
TRI 50c control	20	5	5
Calcium salt	20	5	5
Potassium salt comparator	20	5	5

Approximately 0.6mL of blood was collected *via* a tail vein into 3.8% tri sodium citrate tubes approximately 48 hours prior to dosing and again at 0.5, 1, 2, 4 and 8 hours post-dose.

Plasma was prepared by centrifugation at 3000rpm for 10 minutes at 4°C. Plasma was stored frozen (nominally -20°C) prior to analysis in an automated coagulometer.

C. Results

Table 2: Mean thrombin time for intraduodenally dosed rats

Treatment	Dose (mg/kg)	Group mean thrombin time (s ±sd) at time (hour)					
		-48	0.5	1	2	4	8
TRI 50c control	20	21.3 ±2.69	42.1 ±19.54	27.5 ±9.42	23.5 ±6.40	21.8 ±2.33	21.5 ±2.67
Calcium salt	20	21.6 ±1.77	42.0 ±6.74	34.0 ±1.89	22.6 ±5.10	24.4 ±2.41	22.4 ±1.73
Potassium salt comparator	20	20.0 ±1.92	26.5 ±3.64	24.4 ±3.35	23.2 ±0.83	23.2 ±2.36	21.6 ±0.70

sd = standard deviation

EXAMPLE 17 – ORAL ABSORPTION IN RAT

A. Preparation of Liquid Formulations of TRI50c and Salt

The procedure of example 16 was followed.

B. Oral Studies

The per-oral studies were performed using male Wistar rats, approximately 8 weeks of age and weighing between 250 and 300 g.

Food was withheld overnight prior to dosing and returned approximately 2 hours post-dose. Water was available *ad libitum.*

Each animal received a single administration of control or test article by oral gavage, using a constant dose volume of 4mL/kg.

Individual dose volumes were based on individual body weights, obtained on the day of dosing.

Treatments employed for the study were as follows:

Treatment	Dose level (mg/kg)	Formulation concentration (mg/mL)	Number of animals
TRI 50c control	20	5	5
Calcium salt	20	5	5
Potassium salt comparator	20	5	5

Approximately 0.6mL of blood was collected *via* a tail vein into 3.8% tri sodium citrate tubes approximately 48 hours prior to dosing and again at 0.5, 1, 2, 4 and 8 hours post-dose.

Plasma was prepared by centrifugation at 3000rpm for 10 minutes at 4°C. Plasma was stored frozen (nominally -20°C) prior to analysis in an automated coagulometer.

C. Results

Table 3: mean thrombin times in the rat following oral administration

Treatment	Dose (mg/kg)	Group mean thrombin times (s ±sd) at time (hour)					
		-48	0.5	1	2	4	8
TRI 50c control	20	22.9 ±2.28	26.8 ±1.96	23.3 ±3.68	23.9 ±2.25	23.1 ±2.70	25.1 ±0.33
Calcium salt	20	23.4 ±1.25	25.9 ±3.05	25.7 ±1.94	24.3 ±0.98	25.0 ±1.31	22.9 ±3.46
Potassium salt comparator	20	22.0 ±1.40	24.7 ±2.18	24.1 ±1.87	22.9 ±3.29	23.2 ±1.24	23.8 ±1.79

sd = standard deviation

EXAMPLE 18 – INTRADUODENAL VARIATION

The thrombin times determined in example 16 were analysed to determine the standard deviation for increase in thrombin time, expressed as a percentage of the mean value (this is sometimes called the 'coefficient of variation'). The variation for the Ca salt was calculated to be less than for TRI50c, as shown in Table 4 below.

Table 4: Thrombin times in rats dosed intraduodenally

Product	0h	0.5h	increase		
	←Time→				
TRI50c	23.70	40.02	16.32		
	23.10	40.20	17.10		
	16.85	23.60	6.75		
	21.67	62.55	40.88	SD	SD%
		Mean	**20.26**	**14.53**	**71.7%**
Ca Salt	21.97	35.32	13.35		
	18.75	45.98	27.23		
	23.57	37.27	13.70		
	21.57	49.30	27.73	SD	SD%
		Mean	**20.50**	**8.06**	**39.3%**

CONCLUSION

Examples 16 and 17 indicate that multivalent metal salts of boronic acids have a high oral bioavailability involving an unknown technical effect not linked to solubility.

Example 18 indicates that multivalent metal salts of boronic acids have a low variation in oral bioavailability involving an unknown technical effect not linked to solubility.

It is speculated that the technical effects may in some way involve coordination between the boronate group and the metal ion.

EXAMPLE 19 - ORAL ADMINISTRATION IN DOG

The pharmacokinetics (PK) and pharmacodynamics (PD) of TRI50c (free acid) and its calcium salt were studied in beagle dogs following oral administration. Three female and three male dogs were used for each leg of the study. The weight range of the dogs was 8-18 kg.

The PD was measured as thrombin time and APTT using an automated coagulometer. Plasma concentrations were measured using an LCMS/MS method.

The calcium salt and TRI50c were filled into gelatine capsules and enterically coated. The dose was tailored on an individual basis for each dog. Blood samples were taken into tri-sodium citrate as previously at pre dose, 0.5, 1, 1.5, 2, 3, 6, 8, 12, 16 and 24 hours post dose.

A. RESULTS

A.1 TOLERANCE

The TRI50c and the calcium salt were both tolerated well with no adverse events for the total duration of the study.

A.2 CALCIUM SALT

Unexpectedly high mean thrombin-clotting times were noted in dogs receiving the calcium salt. C max was observed three hours post dose with a mean thrombin clotting time of 80.5 seconds (raised from a base line of 15 seconds). There was still elevation of mean thrombin clotting times 8 hours post dose (mean of 20.2 seconds). All dogs responded dynamically following oral administration of the calcium salt, although there was some variability in response. All dogs dosed with the calcium salt achieved peak thrombin clotting times of up to 148 seconds, although the majority of animals (four out of six) achieved at least a four times elevation in peak thrombin time.

A.3 TRI50c

Absorption as estimated by examination of dynamic response (TT) was variable. A peak thrombin time was noted 1.5 hours post dose (34.2 seconds from a base line of 15.4 seconds). Two animals failed to significantly absorb TRI50c as estimated from their dynamic responses.

B. ACTIVATED PARTIAL THROMBOPLASTIN TIMES

There were no significant changes in APTT from base line following administration of TRI50c. There was a very slight mean elevation in APTT at 3 hours following administration of the calcium salt (14.5 seconds to 18 seconds at peak) this rise was deemed not to be clinically relevant.

C. BIOAVAILABILITY

An estimation of bioavailability was achieved by a conversion of thrombin clotting times following administration of the calcium salt to estimated plasma concentrations.

Unexpectedly high absorption of the calcium salt was seen following oral absorption although there was some variability in responses; estimated bioavailaility was up to as high as 50%. TRI50c was also well tolerated orally although the dynamic responses were significantly less than those for the calcium salt.

EXAMPLE 20 - PARTICLE FORM

TRI50c and its calcium salt were investigated by microscopy and X-ray diffraction.

A. Material and methods

A.1 Microscopic Digital Photographs

Microscopic equipment: Leica® Type 090-135.002
Digital Camera: Nikon® Coolpix 990

A.2 X-Ray diffraction

Equipment: Bruker®AX, Typ "DIFFRAC 5000"

B Results

B.1 Microscopic Digital Photographs

Various shapes for the solid powder were detected. No hint of crystallinity was observed.

B.2 X-Ray diffraction

It is evident from the X-ray diffraction patterns that predominantly amorphous modifications are present for the investigated compounds.

C. Conclusion

The microscopic images show that the particles are very coarse. No crystal appearance could be detected which was confirmed by X-ray powder diffraction where no evidence of crystal structures could be detected.

EXAMPLE 21 - TRI50B INHIBITION OF PLATELET PROCOAGULANT ACTIVITY

Platelet pro-coagulant activity may be observed as the increase, in rate of activation of prothrombin by factor Xa in the presence of factor Va upon the addition of platelets pretreated with thrombin, caused by thrombin alone, collagen alone or a mixture of thrombin and collagen. This property is due to an increase in anionic phospholipid on the surface of the platelet with concomitant release of microvesicle from the surface. This is an essential physiological reaction and people whose platelets have reduced ability to generate procoagulant activity (Scott syndrome) show an increased tendency for bleeding.

Method:

Washed platelets were treated with either 1.15nM thrombin, 23µg/ml collagen or a mixture of both at the same concentration at 37^{o}C. TRI50b was added either for 1 minute prior to the addition of activator or immediately after the incubation with activator. Platelet procoagulant activity was determined as described previously (Goodwin C A et al, *Biochem J.* **1995** 8, 308: 15-21).

TRI50b proved to be a potent inhibitor of platelet procoagulant activity with IC_{50}'s as summarised below:

Table 5: Influence of TRI50b on the induction of platelet procoagulant activity by various agonists:

Table 5

Agonist	**Fold acceleration without TRI50b**	**IC50 plus pre-incubation (nM)**	**IC50 without incubation (nM)**
Thrombin	30	8	3000
Collagen	45	200	300
Thrombin/Collagen	110	3	80

Table 5 records, for example, that when platelets were treated with thrombin they caused a 30-fold acceleration of the rate of activation of prothrombin in comparison with control platelets. Treatment with TRI50 reduced such acceleration by half at the various TRI50 concentration levels given. The significant potency of TRI50 is evidenced by the fact that the IC_{50} values are in the nanomolar range.

TRI50b does not have an effect on ADP, collagen or epinephrine induced aggregation of washed platelets.

EXAMPLE 22 - RABBIT EXTRACORPOREAL SHUNT MODEL

Introduction

The technique describes an animal model in which a platelet rich thrombus is produced. The activity of TRI50b and heparin are compared.

The carotid artery and jugular vein of anaesthetised rabbits were used to create an extracorporeal circuit containing a suspended foreign surface (silk thread). Thrombus deposition is initiated by creation of high sheer stress turbulent arterial blood flow, platelet activation, followed by coagulation in the presence of thrombogenic surfaces. Histopathological studies have shown that the thrombus is platelet rich.

Materials and Methods

Animals:

NZW rabbits (males 2.5-3.5 kg) were used. The animals were allowed food and water up to the induction of anaesthesia.

Anaesthesia:

Animals were premedicated with fontanel/fluanisone (Hypnorm) 0.15 ml total by intramuscular injection. General anaesthesia was induced with methohexitone (10 mg/ml) to effect, followed by endotracheal intubation. Anaesthesia was maintained with isoflurane (1-2.0 %) carried in oxygen /nitrous oxide.

Surgical Preparation:

The animals were placed in dorsal recumbency and the ventral cervical region prepared for surgery. The left carotid artery and right jugular vein were exposed. The artery was cannulated with a large Portex® catheter (yellow gauge), cut to a suitable length. The vein was cannulated with a Silastic® catheter. The shunt comprised of a 5 cm length of 'auto analyser' line (purple /white gauge). Joins to the shunt on the arterial side were made with intermediate size Silastic® tubing. The shunt was filled with saline before exposure to the circulation. The right femoral artery was cannulated for the measurement of blood pressure.

Thread Preparation and insertion:

The central section of the shunt contained a thread 3 centimetres in length. This consisted of 000 gauge Gutterman sewing silk so as to give four strands with a single knot at the end. (The knot section was outside the shunt).

Blood Flow

Blood flow velocity was determined by use of 'Doppler' probes (Crystal Biotech). A silastic probe was positioned over the carotid artery at the point of insertion of the arterial catheter. Flow was recorded on a chart recorder using heat sensitive paper.

RESULTS

Table 6

TREATMENT	DOSE	THROMBUS WEIGHT AFTER 20 minute run	ANTITHROMBOTIC ACTIVITY
Control	N/A	22.4 ±2.2 mg (n=5)	
TRI50b	10mg/kg iv	9.78 ±1.9 mg(n=5)	Active
	3.0mg/kg iv	15.3 ±2.2 mg(n=5)	Active
HEPARIN	100 u/kg iv	22.9 ±1.65 mg(n=4)	Inactive
	300 u/kg iv	10.5 ±1.4 mg (n=4)	Active (Severe bleeding)

Discussion

Table 6 shows that, under high arterial shear conditions, a TRI50b dose of 3mg/kg to 10mg/kg iv significantly inhibits thrombus formation without bleeding, whereas a heparin dose within the normal clinical range for treating venous thrombosis (100u/kg iv heparin) was ineffective. The higher dose of heparin, though active, caused severe bleeding. These results, which show TRI50b effectively inhibiting arterial thrombosis without causing bleeding, are consistent with

TRI50b inhibiting platelet procoagulant activity. In ~~contrast, the thrombin~~ inhibitor heparin, when administered at an approximately equi-effective dose (in terms of inhibition of arterial thrombosis), produced the severe bleeding normal when thrombin inhibitors are used to treat arterial thrombosis.

EXAMPLE 23 – COMPARISON OF BLEEDING TIMES

The aim of the study was to compare the bleeding times of heparin with TRI50b in a suitable model. It is accepted that heparin is a poor inhibitor of platelet procoagulant activity (*J. Biol. Chem.* **1978** Oct 10; 253(19):6908-16; Miletich JP, Jackson CM, Majerus PW1: *J. Clin. Invest.* **1983** May; 71(5):1383-91).

Bleeding times were determined in a rat tail bleeding model following intravenous administration of heparin and TRI50b. The doses employed were chosen on the basis of their efficacy in the rat Wessler and dynamic models and were as follows:

TRI50b:	5 and 10 mg/kg
Heparin:	100 units/kg

MATERIALS AND METHODS

Anaesthesia

Rats were anaesthetised with sodium pentabarbitone at 60 mg/kg (2.0 ml/kg of 30 mg/ml solution by ip. injection). Supplemental anaesthetic was given ip. as required.

Surgical preparation

A jugular vein was cannulated for the administration of test compound. The trachea was also cannulated with a suitable cannula and the animals allowed to breathe 'room air' spontaneously.

Compound administration

These were given in the appropriate vehicle at 1.0 ml/kg intravenously. Heparin was administered in saline, whilst TRI50b was dissolved in ethanol, and then the resultant solution added to water for injection (1 part ethanol to 5 parts water).

Technique

Two minutes following compound administration the distal 2mm of the animal's tail was sectioned with a new scalpel blade and the tail immersed in warm saline (37°C) contained in a standard 'universal' container, so that the blood stream was clearly visible. The bleeding time recording was started immediately following transection until the cessation of blood flow from the

tip of the tail. A period of 30 seconds was allowed after the blood flow from the tail had stopped to ensure that bleeding did not re-commence, if bleeding did start again the recording time was continued for up to a maximum of 45 minutes.

Results

Table 7 gives a summary of the bleeding results and shows the increases above base line values.

Table 7

Summary table of bleeding results

Treatment	Bleeding time min (± SEM†)
Saline	5.1 ± 0.6
Heparin 100 u/kg iv	>40*
TRI50b 5 mg/kg iv	11.3 ± 1.2
TRI50b 10 mg/kg iv	30.4 ± 5.2

*Severe bleeding in all animals, with no cessation after 40 minutes.

†SEM = standard error of the mean

Discussion

The results show that TRI50b was superior to heparin (produced less bleeding) at all doses. It should be noted that when 100 u/kg heparin is compared with 5 mg/kg TRI50b, heparin-treated animals bled more extensively than those receiving TRI50b; it was previously established (Example 22) that heparin at a dose of 100 u/kg is a less effective inhibitor of arterial thrombosis than TRI50b at a dose of 3.0 mg/kg. Heparin is primarily a thrombin inhibitor and a poor inhibitor of platelet procoagulant activity; the results are therefore consistent with TRI50b exerting anti-coagulant activity by inhibition of platelet coagulant activity in addition to thrombin inhibiting activity.

EXAMPLE 24 - TRI50B AS A PRODRUG FOR TRI50C: PHARMACOKINETICS AND ABSORPTION

MATERIALS AND METHODS

Animals

Rats, body weight circa 250-300g were used. The animals were fasted only on the day of use for the iv stage. Animals were fasted on the night prior to study for the oral and intraduodenal studies, water was allowed up to the time of anaesthesia.

Table 8:

iv phase

Treatment	Dose mg/kg iv	n
TRI50b	1.0mg/kg	3
TRI50c	1.0mg/kg	3

Table 9:

oral phase

Treatment	Dose mg/kg po	n
TRI50b	20mg/kg	2
TRI50c	20mg/kg	2

Table 10:

intraduodenal phase

Treatment	Dose mg/kg po	n
TRI50b	20mg/kg	3
TRI50c	20mg/kg	3

Dose

Formulation (TRI50b/TRI50c)

These were dosed in a formulation prepared as follows: 48 mg/ml of TRI50b is dissolved in ethanol: PEG 300 (2:3 vol: vol). Just before administration, 5 volumes of this solution is mixed with 3 volumes of 5% kollidon 17 8F.

i.v. Phase

Both compounds were given at a dose of 1.0mg/kg iv.

Oral Phase

1) Both compounds were dosed by oral gavage at 20mg/kg.

2) As 1) but directly into the duodenum.

The compounds were dosed in a PEG/ethanol/kollidon formulation which was prepared immediately before, as described immediately under the heading "Dose": Stock 15.0mg/ml. This was dosed at 1.33ml/kg (equivalent to 30mg/kg).

Methods

Oral gavage

Rats were dosed at 20mg/kg. Approximately 30 minutes following dosing the rats were anaesthetised.

Intraduodenal administration

The compounds were instilled directly into the duodenum after anaesthesia and surgical procedures had been completed.

Blood sampling

i.v. Phase

A pre dose sample was taken followed by: 0, 2, 5, 10, 20, 30, 40, 60 and 90 minutes post dose.

Oral phase

Blood (0.81ml) was taken from the carotid cannula into (0.09ml) of 3.8% w/v tri sodium citrate following anaesthesia and surgery. The first samples were taken one-hour post dose. Then at, 1.5, 2, 4 hours post dose.

Intraduodenal phase

Blood samples were taken: Pre dose, then at 0.25, 0.5, 0.75, 1.0, 2, 3 and 4 hours post dosing.

Plasma

This was obtained by centrifugation (3000 RPM for 10 min) and stored at -20°C prior to analysis.

RESULTS

PHARMACOKINETIC ANALYSIS

Intravenous phase

Table 11:

i.v. pharmacokinetic data

	TRI50b	TRI50c
Elimination half life: minutes	35 minutes	36.6 minutes
Area under curve	1.68	1.48
Mean Residence Time	46 minutes	45 minutes
Clearance: ml/min/kg	10	11.3
Volume Distribution Litres/kg	0.5	0.59
Max Plasma Concentration (observed)	2.24	2.35

The following results are represented in Figures 3 to 5:

Fig 3: intravenous phase clearance and kinetics following a single dose of TRI50b or its free acid (TRI50c). The figure shows the observed assay data.

Fig 4: oral phase clearance and kinetics following dosing with TRI50b or its free acid (TRI50c).

Fig 5: oral phase clearance and kinetics following intraduodenal dosing with TRI50b or its free acid (TRI50c).

CONCLUSION

When given by the intraduodenal route TRI50b achieved a higher bioavailability (peak plasma concentration) than the free acid. The i.v. kinetics were similar for both compounds. The data are consistent with TRI50b being rapidly hydrolysed in plasma to TRI50c and with TRI50c being the active principle.

Taken together with the data from examples 16 to 19, the results of examples 21 to 24 indicate that oral administration of TRI50c as the calcium salt will provide an excellent way to treat arterial thrombosis and/or venous thrombosis.

EXAMPLE 25 – Human Clinical Studies

In human clinical volunteer studies with doses of up to 2.5mg/kg i.v. (dosages which significantly prolong the thrombin clotting time), TRI50b had no effect on Simplate bleeding time (i.e. bleeding time measured using a Simplate® bleeding time device).

It will be appreciated from the foregoing that the invention provides boronic acid salts useful for pharmaceutical purposes and which feature one or more of the following attributes: (1) improved amount of oral bioavailability; (2) improved consistency of oral bioavailability; (3) improved stability; and (4), in any event, not suggested by the prior art.

The selection of active ingredient for a pharmaceutical composition is a complex task, which requires consideration not only of biological properties (including bioavailability) but also of physicochemical properties desirable for processing, formulation and storage. Bioavailability itself is dependent on various factors, often including in vivo stability, solvation properties and absorption properties, each in turn potentially dependent on multiple physical, chemical and/or biological behaviours.

CLAIMS

1. A salt of a pharmaceutically acceptable multivalent (at least divalent) metal and an organoboronic acid drug (where the term "drug" embraces prodrugs).

2. A salt of claim 1 wherein the metal is a Group II or Group III metal or zinc.

3. A salt of claim 1 or claim 2 wherein the metal is divalent.

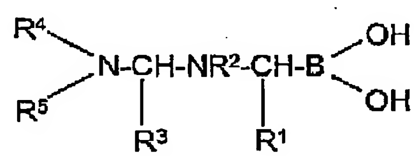
4. A salt of claim 1 wherein the metal is calcium.

5. A salt of claim 1 wherein the metal is magnesium.

6. A salt of any of claims 1 to 5 wherein the organoboronic acid is hydrophobic.

7. A salt of any of claims 1 to 6 wherein the organoboronic acid comprises a boropeptide or boropeptidomimetic.

8. A salt of any of claims 1 to 6 wherein the organoboronic acid is of the formula (I):



(I)

where:

R^1 is H or a non-charged side group;

R^2 is H or C_1-C_{13} hydrocarbyl optionally containing in-chain oxygen or sulfur and optionally substituted by a substituent selected from halo, hydroxy and trifluoromethyl;

or R^1 and R^2 together form a C_1-C_{13} moiety which in combination with N-CH forms a 4-6 membered ring and which is selected from alkylene (whether branched or linear) and alkylene containing an in-chain sulfur or linked to N-CH through a sulfur;

R^3 is the same as or different from R^1 provided that no more than one of R^1 and R^2 is H, and is H or a non-charged side group;

R^4 is H or C_1-C_{13} hydrocarbyl optionally containing in-chain oxygen or sulfur and optionally substituted by a substituent selected from halo, hydroxy and trifluoromethyl;

or R^3 and R^4 together form a C_1-C_{13} moiety which in combination with N-CH forms a 4-6 membered ring and which is selected from alkylene (whether branched or linear) and alkylene containing an in-chain sulfur or linked to N-CH through a sulfur; and

R^5 is X-E- wherein E is nothing or a hydrophobic moiety selected from the group consisting of amino acids (natural or unnatural) and peptides of two or more amino acids (natural or unnatural) of which more than half are hydrophobic and X is H or an amino-protecting group.

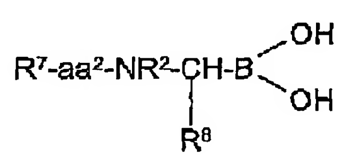
9. A salt of claim 8 where R^2 and R^4 are H, or R^2 is H and R^3 and R^4 together form a said C_1-C_{13} moiety.

10. A salt of claim 8 or claim 9 where hydrocarbyl is selected from the group consisting of alkyl; alkyl substituted by cycloalkyl, aryl or heteroaryl; cycloalkyl; aryl; and heteroaryl.

11. A salt of any of claims 8 to 10 wherein E is nothing.

12. A salt of any of claims 8 to 10 wherein E is a hydrophobic amino acid.

13. A salt of any of claims 1 to 6 wherein the organoboronic acid is of the formula (II):



(II)

wherein

R^7 is X-E'- wherein X is hydrogen or an amino-protecting group and E' is absent or is a hydrophobic amino acid;

R^8 is an optionally substituted moiety containing from 1 to 4 carbon atoms selected from the group consisting of alkyl, alkoxy and alkoxyalkyl, the optional substituents being hydroxy and halogen (F, Cl, Br, I); and

aa^2 is a hydrophobic amino acid.

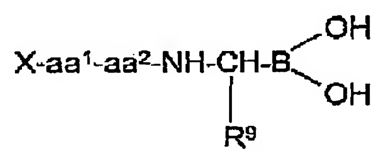
14. A salt of any of claims 8 to 13 where X is R^6-$(CH_2)_p$-C(O)-, R^6-$(CH_2)_p$-$S(O)_2$-, R^6-$(CH_2)_p$-NH-C(O)- or R^6-$(CH_2)_p$-O-C(O)- wherein p is 0, 1, 2, 3, 4, 5 or 6 and R^6 is H or a 5 to 13-membered cyclic group optionally substituted by 1, 2 or 3 substituents selected from halogen, amino, nitro, hydroxy, a C_5-C_6 cyclic group, C_1-C_4 alkyl and C_1-C_4 alkyl containing, and/or linked to the cyclic group through, an in-chain O, the aforesaid alkyl groups optionally being substituted by a substituent selected from halogen, amino, nitro, hydroxy and a C_5-C_6 cyclic group.

15. A salt of claim 14 wherein said 5 to 13-membered cyclic group is aromatic or heteroaromatic.

16. A salt of claim 15 wherein said 5 to 13-membered cyclic group is phenyl or a 6-membered heteroaromatic group.

17. A salt of any of claims 14 to 16 wherein X is R^6-$(CH_2)_p$-C(O)- or R^6-$(CH_2)_p$-O-C(O)- and p is 0 or 1.

18. A salt of a pharmaceutically acceptable multivalent metal and a peptide boronic acid of formula (III):



(III)

where:

X is H (to form NH_2) or an amino-protecting group;

aa^1 is Phe, Dpa or a wholly or partially hydrogenated analogue thereof;

aa^2 is an imino acid having from 4 to 6 ring members;

R^9 is a group of the formula $-(CH_2)_m-W$, where m is 2, 3 or 4 and W is –OH, –OMe, –OEt or halogen (F, Cl, Br or I).

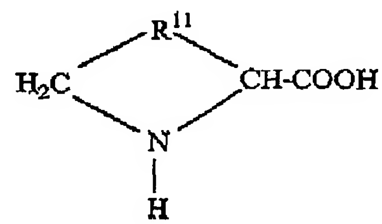
19. A salt of claim 18 wherein aa^1 is selected from Dpa, Phe, Dcha and Cha.

20. A salt of claim 18 or claim 19 wherein aa^1 is of R-configuration.

21. A salt of claim 20 wherein aa^1 is (R)-Phe (that is, D-Phe) or (R)-Dpa (that is, D-Dpa).

22. A salt of claim 20 wherein aa^1 is (R)-Phe.

23. A salt of any of claims 18 to 22 wherein aa^2 is a residue of an imino acid of formula (IV)



(IV),

where R^{11} is $-CH_2-$, CH_2-CH_2-, $-S-CH_2-$ or $-CH_2-CH_2-CH_2-$, which group, when the ring is 5- or 6- membered, is optionally substituted at one or more $-CH_2-$ groups by from 1 to 3 C_1-C_3 alkyl groups.

24. A salt of claim 23 wherein aa^2 is of S-configuration.

25. A salt of claim 18 wherein aa^2 is a natural proline residue.

26. A salt of claim 18, wherein aa^1-aa^2 is (R)-Phe-(S)-Pro (that is, D-Phe-L-Pro).

27. A salt of any of claims 18 to 26 wherein R^9 is 2-bromoethyl, 2-chloroethyl, 2-methoxyethyl, 3-bromopropyl, 3-chloropropyl or 3-methoxypropyl.

28. A salt of any of claims 18 to 26 wherein R^9 is 3-methoxypropyl.

29. A salt of any of claims 18 to 27 wherein X is $R^{6'}-(CH_2)_p-C(O)-$ or $R^{6'}-(CH_2)_p-O-C(O)-$, where $R^{6'}$ is phenyl or a 6-membered heteroaromatic group and p is 0 or 1.

30. A salt of any of claims 18 to 27 wherein X is benzyloxycarbonyl.

31. A salt of claim 18 which is a ~~salt of a~~ compound of formula (VIII):

X-(R)-Phe-(S)-Pro-(R)-Mpg-$B(OH)_2$ (VIII).

32. A salt of any of claims 18 to 31 which is a divalent metal salt of the peptide boronic acid.

33. A salt of claim 32 wherein the metal is calcium.

34. A salt of claim 32 wherein the metal is magnesium.

35. A salt of any of claims 18 to 31 which is a Group III metal salt of the peptide boronic acid.

36. A salt of claim 35 wherein the metal is aluminium.

37. A salt of claim 35 wherein the metal is gallium.

38. A salt of any of claims 1 to 37 which is an acid salt (that is, wherein one B-OH group remains protonated).

39. A salt of any of claims 1 to 38 wherein the salt comprises a boronate ion derived from the boronic acid and a counterion and wherein the salt consists essentially of a salt having a single type of counterion.

40. A product obtainable by (having the characteristics of a product obtained by) reaction of a boronic acid as defined in any of claims 1 and 6 to 31 and a pharmaceutically acceptable multivalent (at least divalent) metal base.

41. A product obtainable by (having the characteristics of a product obtained by) reaction of a boronic acid as defined in any of claims 1 and 6 to 31 and a pharmaceutically acceptable multivalent (at least divalent) metal hydroxide.

42. A product of claim 40 or claim 41 wherein the metal is as defined by any of claims 2 to 5.

43. A product of any of claims 40, 41 and 42 wherein the reaction comprises combining a solution of the peptide boronic acid in a water-miscible organic solvent with an aqueous solution of the base, allowing the acid and the base to react at ambient temperature (e.g. at a temperature of from 15 to 25°C), evacuating the reaction mixture to dryness, redissolving the salt in water, filtering the resulting solution and drying it, and, if required, removing at least a

portion of the residual water by further redissolution in ethyl acetate or THF followed by evaporating to dryness.

44. A product of claim 43 wherein the acid and the base are allowed to react for at least one hour.

45. A product of claim 43 or claim 44 wherein the water-miscible organic solvent is acetonitrile or an alcohol, e.g. ethanol, methanol, a propanol, especially iso-propanol, or another alkanol, or a mixture of alcohols.

46. A product of any of claims 40 to 45 wherein the boronic acid is as defined by any of claims 18 to 31.

47. A method for drying a boronic acid salt, comprising dissolving it in ethyl acetate and then evaporating the resultant solution to dryness.

48. A pharmaceutical formulation in oral dosage form comprising a salt of any of claims 1 to 39 or a product of any of claims 40 to 45 and a pharmaceutically acceptable diluent, excipient or carrier.

49. A pharmaceutical formulation of claim 48 which is adapted to release the salt or the product in the duodenum.

50. A pharmaceutical composition of claim 49 which is enterically coated.

51. A method of inhibiting thrombin in the treatment of disease comprising orally administering to a mammal a therapeutically effective amount of an active agent selected from the group consisting of salts of any of claims 18 to 37 and a product of claim 46.

52. A method of claim 51 wherein the active agent is in a formulation adapted to release the active agent in the duodenum.

53. The use of a salt of any of claims 18 to 37 or a product of claim 46 for the manufacture of an oral medicament for treating thrombosis.

54. The use of a peptide boronic acid of formula (III) as defined in any of claims 18 to 37 as an intermediate to make a salt of any of claims 18 to 37 or a product of claim 46.

55. A method of preparing a salt of any of claims 1 to 39 or a product of any of claims 40 to 46, comprising contacting a peptide boronic acid of formula (III) as defined in claim 18 with a base capable of making such a salt.

56. A method of preparing a salt of any of claims 1 to 39 or a product of any of claims 40 to 46, comprising mixing together a solution of an alkali metal salt of an organoboronic acid drug and a solution of a multivalent metal salt, and recovering the respective multivalent metal salt of the organoboronic acid.

57. A method of claim 56, wherein the organoboronic acid drug is as defined in any of claims 13 to 17 or 18 to 31.

58. A method of claim 56 or claim 57, wherein the multivalent metal is zinc.

59. A method of claim 56 or claim 57, wherein the multivalent metal is calcium.

60. A method of any of claims 56 to 59, wherein the alkali metal is sodium.

61. A method of any of claims 56 to 60 wherein the multivalent metal salt of the organoboronic acid is recovered by allowing it to precipitate and separating the solid precipitate from the reaction solution.

62. The use of the sodium salt of a compound of Formula III as defined in any of claims 18 to 31 as starting material to prepare the corresponding calcium or zinc salt.

63. A sodium or potassium salt of a compound of Formula III as defined in any of claims 18 to 31.

64. A salt of claim 63 when in aqueous solution.

65. A method of treating venous and/or arterial thrombosis by prophylaxis or therapy, comprising administering to a mammal suffering from, or at risk of suffering from, arterial thrombosis a therapeutically effective amount of a product selected from a salt of any of claims 18 to 31 and a product of claim 46.

66. A method of claim 65 wherein the disease is an acute coronary syndrome.

67. A method of inhibiting platelet procoagulant activity, comprising administering to a mammal at risk of, or suffering from, arterial thrombosis a therapeutically effective amount of a product selected from a salt of any of claims 18 to 31 and a product of claim 46.

68. A method of claim 67 wherein the disease is an acute coronary syndrome.

69. A method of treating by way of therapy or prophylaxis an arterial disease selected from acute coronary syndromes, cerebrovascular thrombosis, peripheral arterial occlusion and arterial thrombosis resulting from atrial fibrillation, valvular heart disease, arterio-venous shunts, indwelling catheters or coronary stents, comprising administering to a mammal a therapeutically effective amount of a product selected from a salt of any of claims 18 to 31 and a product of claim 46.

70. A method of claim 69 wherein the disease is an acute coronary syndrome.

71. The use of a product selected from a salt of any of claims 18 to 31 and a product of claim 46 for the manufacture of an oral medicament for treating arterial thrombosis.

72. The use of a salt of any of claims 18 to 31 or a product of claim 46 for the manufacture of an oral medicament for treating by way of therapy or prophylaxis a disease selected from acute coronary syndromes, cerebrovascular thrombosis and peripheral arterial occlusion.

73. The use of claim 72 wherein the medicament is for treating an acute coronary syndrome.

74. The use, for the manufacture of a medicament for treating in a mammalian subject by way of therapy or prophylaxis a disease selected from acute coronary syndromes, cerebrovascular thrombosis, peripheral arterial occlusion and arterial thrombosis resulting from atrial fibrillation, valvular heart disease, arterio-venous shunts, indwelling catheters or coronary stents, of a salt of any of claims 18 to 31 or a product of claim 46.

75. The use of a salt of any of claims 18 to 31 or a product of claim 46 for the manufacture of a medicament for inhibiting platelet procoagulant activity.

P41074GB1.4 (as filed) - Multivalent metal salts I

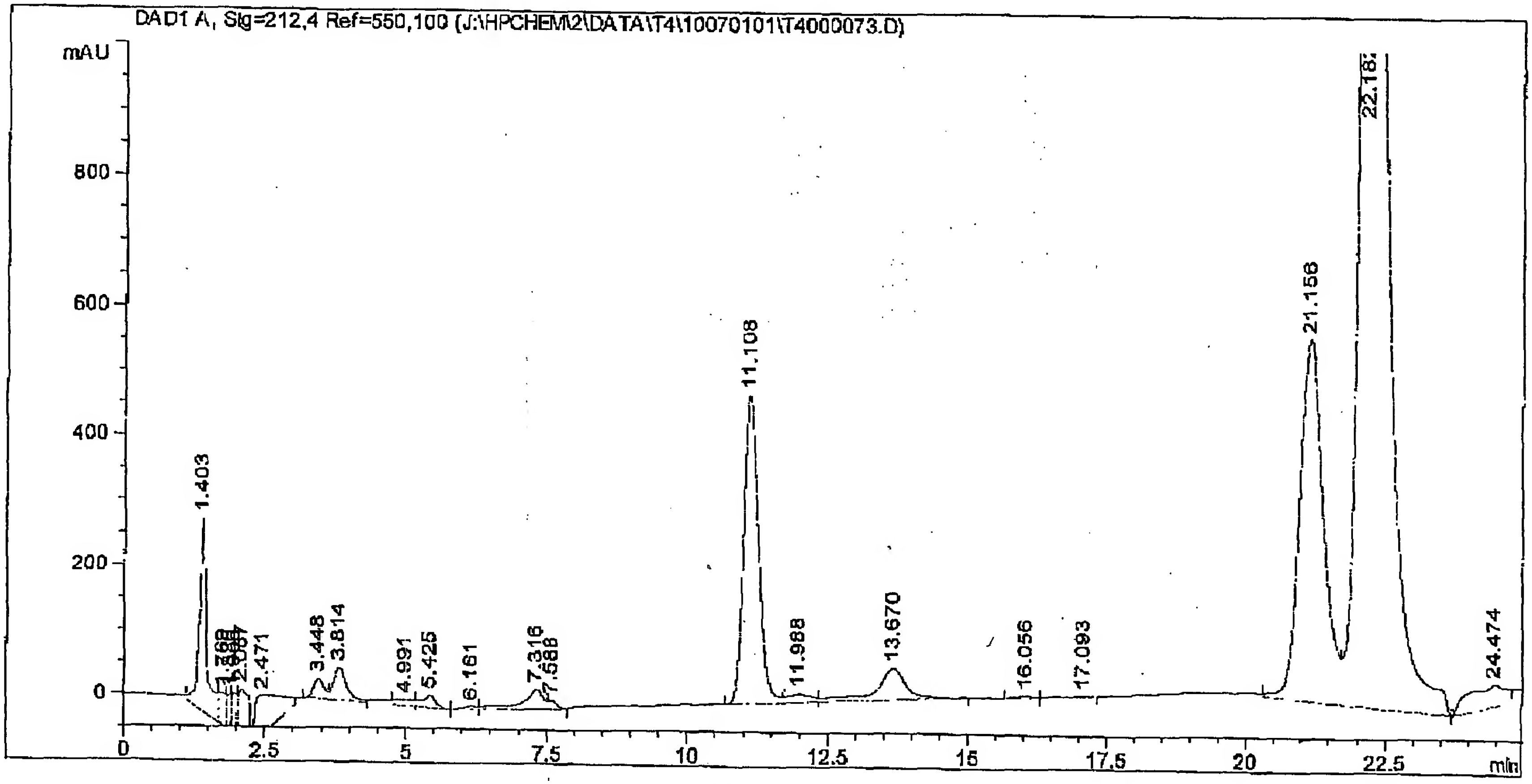


Figure 1: Assay method

1/4

Tri50b(I)	Rt = 11.1
Tri50b(II)	Rt = 13.7
Tri50c(I)	Rt = 21.2
Tri50c(II)	Rt = 22.2

P41074GB1.4 (as filed) - Multivalent metal salts I

2/4

Re-fozen thrombin aliquot and active compounds

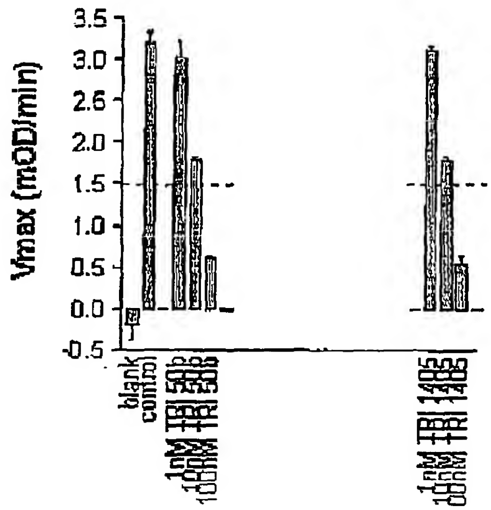


Fig 2

Fig 3

3/4

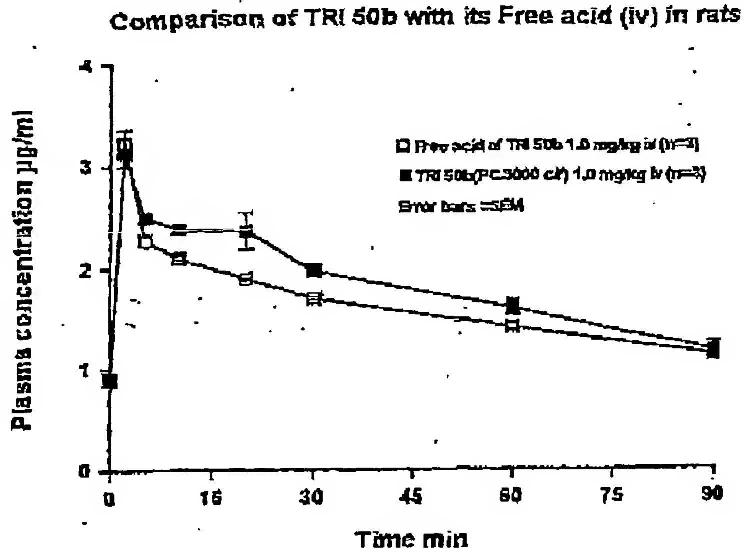

Comparison of TRI 50b with its Free acid (iv) in rats
Plasma concentration µg/ml
Time min
0
15
30
45
60
75
90
1
2
3
4
Free acid of TRI 50b 1.0 mg/kg iv (n=3)
TRI 50b(PC3000 cit) 1.0 mg/kg iv (n=3)
Error bars =SEM

Fig 4

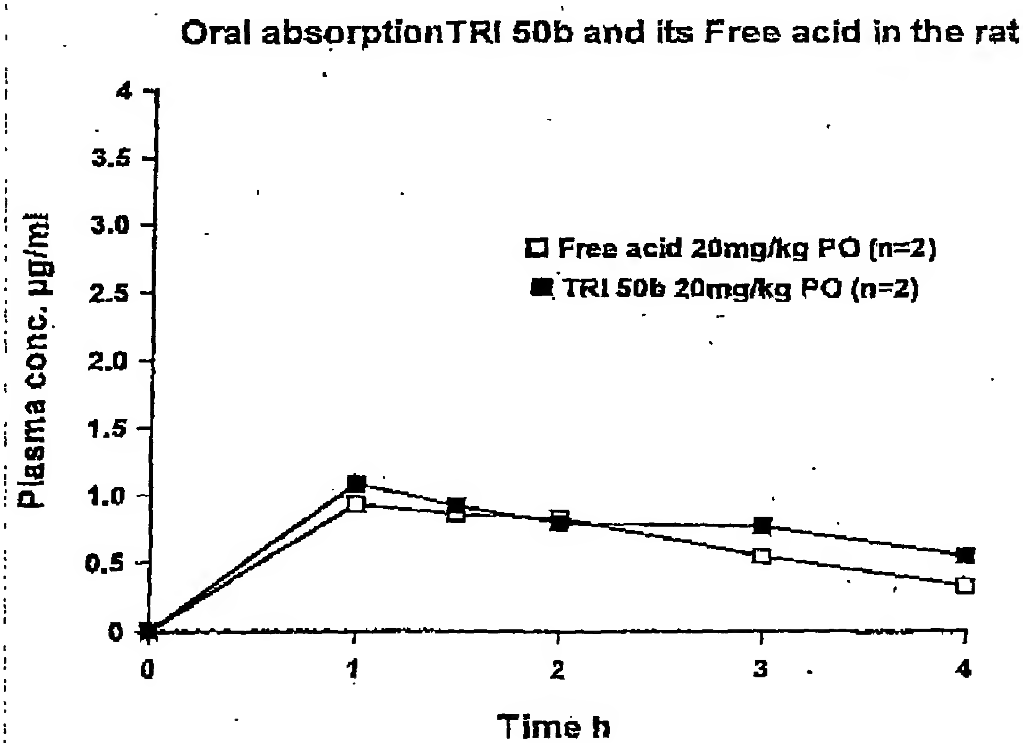
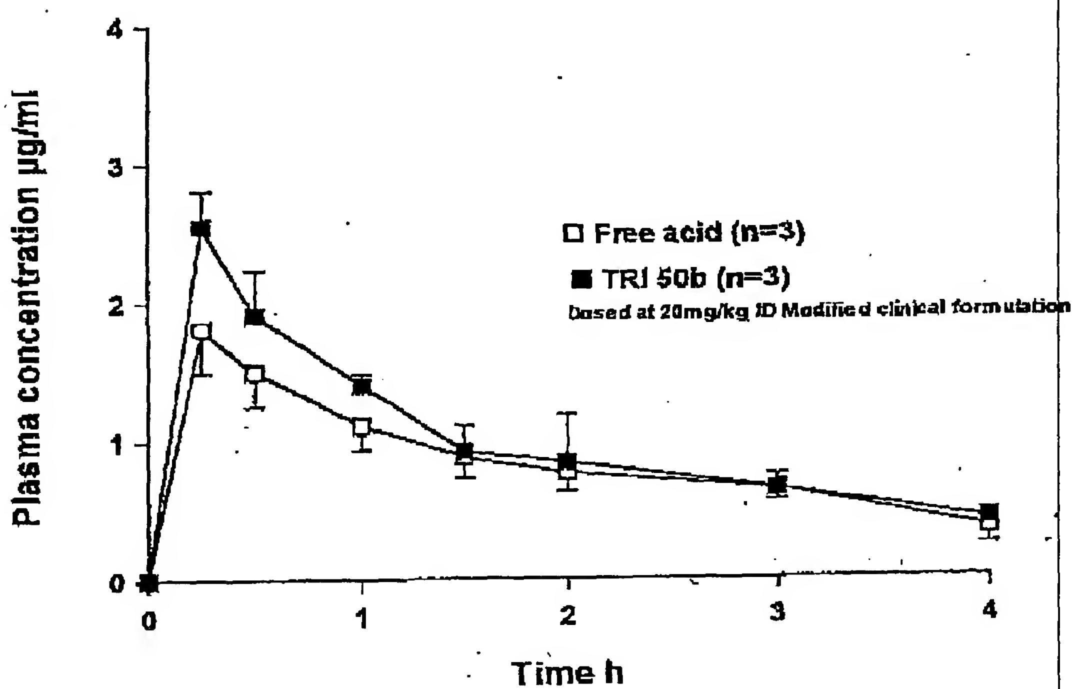

Oral absorptionTRI 50b and its Free acid in the rat
Plasma conc. µg/ml
Time h
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4
1
2
3
Free acid 20mg/kg PO (n=2)
TRI 50b 20mg/kg PO (n=2)

Fig 5



Plasma concentration µg/ml
4
3
2
1
0
□ Free acid (n=3)
■ TRI 50b (n=3)
Dosed at 20mg/kg ID Modified clinical formulation
0
1
2
3
4
Time h